

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

NIPPON SHINYAKU CO., LTD.,	)	
Plaintiff,	)	
	)	C.A. No. 21-1015 (JLH)
v.	)	
	)	<b>DEMAND FOR JURY TRIAL</b>
SAREPTA THERAPEUTICS, INC.,	)	
Defendant.	)	
<hr/>		
SAREPTA THERAPEUTICS, INC. and THE	)	
UNIVERSITY OF WESTERN AUSTRALIA,	)	
Defendant/Counter-Plaintiffs,	)	
	)	
v.	)	
	)	
NIPPON SHINYAKU CO., LTD. and NS	)	
PHARMA, INC.,	)	
Plaintiff/Counter Defendants.	)	

## DEMAND FOR JURY TRIAL

SAREPTA THERAPEUTICS, INC. and THE UNIVERSITY OF WESTERN AUSTRALIA,  
Defendant/Counter-Plaintiffs,

v.

NIPPON SHINYAKU CO., LTD. and NS PHARMA, INC.,  
Plaintiff/Counter Defendants.

## EXHIBIT 14A

**NIPPON SHINYAKU CO., LTD. AND NS PHARMA, INC.’S MOTION *IN LIMINE*  
NO. 1 TO PRECLUDE IMPROPER RELIANCE ON POST-PRIORITY DATE  
EVIDENCE TO SUPPORT THE UWA PATENTS’ VALIDITY**

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NIPPON SHINYAKU CO., LTD., Plaintiff,	)	
v.	)	C.A. No. 21-1015 (JLH)
SAREPTA THERAPEUTICS, INC., Defendant.	)	<b>DEMAND FOR JURY TRIAL</b>
SAREPTA THERAPEUTICS, INC. and THE UNIVERSITY OF WESTERN AUSTRALIA, Defendant/Counter-Plaintiffs,	)	[REDACTED]
v.	)	[REDACTED]
NIPPON SHINYAKU CO., LTD. and NS PHARMA, INC., Plaintiff/Counter Defendants.	)	

**NIPPON SHINYAKU CO., LTD. AND NS PHARMA, INC.’S MOTION *IN LIMINE*  
NO. 1 TO PRECLUDE IMPROPER RELIANCE ON POST-PRIORITY DATE  
EVIDENCE TO SUPPORT THE UWA PATENTS’ VALIDITY**

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Nippon Shinyaku Co., Ltd. and Counterclaim  
Defendant NS Pharma, Inc.*

**Dated: April 19, 2024**

Sarepta and UWA seek to improperly rely on post-priority date testing as evidence of the later state of the art: that “the art with respect to ASOs targeting the exon 53 hot spot became predictable” through the asserted UWA Patents’ experimental work. D.I. 469 at 13, 15. As Dr. Dowdy, the sponsor for much of this evidence, explains, his alleged “expectation” of predictability is “premised on the Wilton 2005 patent,” but “*proven by all the real world data subsequently.*” Ex. 1, Dowdy Dep. at 69:14-70:9, 192:1-16 (emphasis added). Such reliance on the post-priority date art to “confirm” written description, enablement, and supposed predictability is barred by Federal Circuit law. *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1374 (Fed. Cir. 2017) (“post-priority-date evidence proffered to illuminate the post-priority-date state of the art [] is improper”) (citing *In re Hogan*, 559 F.2d 595 (CCPA 1977)). The Court should preclude Sarepta and UWA under Fed. R. Evid. 402 and 403 from adducing post-priority date evidence as alleged support for the UWA Patents’ validity or arguing the same. The resulting juror confusion regarding the proper legal analysis and unfair prejudice to NS merits this relief.

Written description and enablement are determined as of the priority date. *Id.* at 1373, 1375. As a general rule, post-priority evidence is “legally irrelevant” to these priority-date inquiries. *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1355 (Fed. Cir. 2010). The Federal Circuit’s only recognized exceptions involve using post-priority date as evidence of *invalidity* for genus claims. In *Amgen*, the Federal Circuit endorsed using later-developed, dissimilar species “to show that a patent does not disclose a representative number of species of a claimed genus” or “common structural features.” 872 F.3d at 1375. And it likewise endorsed defendants using post-priority date evidence of their own “lengthy and potentially undue experimentation” as evidence of non-enablement. *Id.* Neither apply here.

Courts have repeatedly rejected patentees’ reliance on supposedly confirmatory post-

priority date evidence. *Ariad*, 598 F.3d at 1355 (patentee’s evidence “directed to the later [priority] date” not chosen by jurors is “legally irrelevant”); *Juno Therapeutics, Inc. v. Kite Pharma, Inc.*, 10 F.4th 1330, 1341 (Fed. Cir. 2021) (inventors’ “testimony about post-priority date developments” where he made additional species was “irrelevant”); *Biogen Int’l GMBH v. Mylan Pharms. Inc.*, 18 F.4th 1333, 1343-44 (Fed. Cir. 2021) (“That Biogen later established the therapeutic efficacy of DMF480 is of no import”); *MorphoSys AG v. Janssen Biotech, Inc.*, 358 F.Supp. 3d 354, 367 (D. Del. 2019) (structural motif identified using post-priority date knowledge impermissibly illuminated the “state of the art subsequent to the priority date”).

Here, Sarepta and UWA may attempt to adduce post-priority date evidence as support for the UWA Patents’ validity through either its fact witnesses (UWA and Sarepta researchers) and/or Dr. Dowdy. Dr. Dowdy’s opinions—which summarize post-priority date work that ostensibly “validates” or “confirms” the predictability he contends arose from the alleged “hot spot”—are illustrative. He describes research by, *inter alia*, UWA and Sarepta and then relies upon “this highly relevant evidence” (¶ 135) as “*validat[ing]* what Dr. Wilton and co-inventors disclosed in June 2005 and *confirm[ing]* that independent researchers recognized the Wilton Patents’ disclosure,” (¶ 94) “*confirm[ing]* the hot spot identified by Dr Wilton,” (¶ 145) and “*illustrat[ing]* that the Wilton Patents resolved this unpredictability” (¶ 266). Ex. 2, Dowdy Reb.; *see also id.* at ¶¶ 92, 96, 115, 119, 122, 126, 128, 134-135, 138, 141.

Dr. Dowdy’s Rebuttal Report makes clear that Sarepta intends to adduce evidence of these later developments in the art to “confirm” validity. *See* Ex. 2, Dowdy Reb. ¶ 31 (the alleged structure-function relationship), ¶ 128 (the “criticality” of the 12 consecutive bases limitation), ¶¶ 210-213 (post-priority data “confirms... written description support”); *see also id.* at ¶¶ 72, 76-88, 91-94, 219, 249, 271. Dr. Dowdy admitted as much. Ex. 1, Dowdy Dep. at 192:5-16.



Adding to the likelihood of juror confusion and unfair prejudice, Dr. Dowdy admits that this post-priority date evidence is *not* commensurate with the “full scope” of the UWA Patents’ claims. Dr. Dowdy admitted that he only reviewed ASOs with 100% complementarity, despite agreeing the claims allow for mismatches and insertions. Ex. 1, Dowdy Dep. at 15:25-16:7, 24:9-23, 187:25-188:8. Further, many of the post-priority date ASOs he relies upon undisputedly do not meet all “structural requirements of the claims.” Ex. 2, Dowdy Reb. ¶¶ 77-78, 83, 85-89.

Importantly, Sarepta and UWA should not be allowed to backdoor these arguments in connection with its obviousness arguments against the NS Patents. Although Sarepta does not assert the UWA researchers’ work in any obviousness combination, Dr. Dowdy’s Opening Report nevertheless discusses the “hot spot” supposedly disclosed by the UWA Patents at length. *See* Ex. 3, Dowdy Op. ¶¶ 102, 417, 446 (“The hot spot identified in 2005 by Dr. Wilton and his colleagues continued to be targeted and refined for the next several years by Dr. Wilton’s group and other independent researchers”); *see also id.* ¶¶ 103-106, 110, 116, 130, 459, 492. Dr. Dowdy then incorporates these sections of his Opening Report into his defense of the UWA Patents’ validity. *See, e.g.,* Ex. 2, Dowdy Reb. at ¶¶ 66, 72, 77, 80, 81, 84, 91, 133, 134, 142, 145, 266.

Sarepta and UWA should be precluded from characterizing post-June 28, 2005 evidence listed in Appendix A as purportedly “validating,” “confirming,” “targeting,” “further evidencing,” etc. an exon 53 hot spot allegedly established by the UWA Patents’ specification. Allowing such testimony would incorrectly suggest to the jury that this post-priority date evidence may be used to support the validity of the UWA Patents when it cannot. In particular, allowing Dr. Dowdy to testify that other researchers “recognized” or “focused” on this supposed “hot spot” when he has no such insight would cloak mere speculation about their motivation under the guise of expert testimony, further prejudicing NS and confusing the jury.

April 19, 2024

Respectfully submitted,

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and Counterclaim Defendant NS  
Pharma, Inc.*

**Appendix A**

<b>Document</b>	<b>Exhibit Nos.</b>
MDA Press Release dated August 12, 2020	DTX-0459
WO 2010/0485586 ("Sazani PCT '586")	JTX0472
WO 2011/057350 ("Wilton PCT '350")	JTX0473
Popplewell et al., "Design of Phosphorodiamidate Morpholino Oligomers (PMOs) for the Induction of Exon Skipping of the Human <i>DMD</i> Gene," Mol. Ther. (2009) 17(3): 554-561 SRPT-VYDS-0008344-55 ("Popplewell 2009")	JTX0276
Popplewell et al., "Comparative Analysis of Antisense Oligonucleotide Sequences Targeting Exon 53 of the Human <i>DMD</i> Gene: Implications for Future Clinical Trials," Neuromuscul. Disord. (2010) 20(2):102-110 ("Popplewell 2010")	JTX0283
U.S. 10,662,217 ("NS's '217 Patent")	JTX0006
US 2010/0168212 ("Popplewell US '212")	PTX0032
WO 2014/100714 ("Bestwick PCT '714")	JTX0474
WO 2014/153240 ("Bestwick PCT '240")	PTX0030
SRPT-VYDS-0201524-88 ("Sequence Selection Report")	JTX0363
SRPT-VYDS-0162285-461 ("Adams Lab Notebook 3")	JTX0344
SRPT-VYDS-0161650-826 ("Adams Lab Notebook 6")	JTX0339
SRPT-VYDS-0161337-480 ("Adams Lab Notebook 8")	JTX0337
SRPT-VYDS-0158291-435 ("Westcott Lab Notebook")	JTX0317
SRPT-VYDS-0161827-956 ("Forrest Lab Notebook 4")	JTX0340
SRPT-VYDS-0161292-336 ("Forrest Lab Notebook 5")	JTX0336
SRPT-VYDS-0160521-639 ("Jiminy Synthesis Lab Notebook 15")	JTX0331
SRPT-VYDS-0228257-67 ("Schnell Decl. 1")	DTX-0745

<b>Document</b>	<b>Exhibit Nos.</b>
SRPT-YDS-0228268-75 ("Schnell Decl. 2)	JTX0471
NS00102924-87 ("0643 CERI Report")	JTX0224
NS00102988-3060 ("0644 CERI Report")	JTX0225
NS00103061-110 ("0661 CERI Report")	JTX0226
Watanabe Ex. 43, p. 41 (Dowdy Reb ¶ 88)	JTX0198

# Exhibit 1 to NS's MIL No. 1

UNITED STATES DISTRICT COURT

DISTRICT OF DELAWARE

NIPPON SHINYAKU CO., LTD.,	)	
	)	
Plaintiff,	)	
	)	
vs.	)	C.A. No.
	)	21-1015(GBW)
SAREPTA THERAPEUTICS, INC.,	)	
	)	
Defendant.	)	
<hr/>		
	)	
SAREPTA THERAPEUTICS, INC., and	)	
THE UNIVERSITY OF WESTERN	)	
AUSTRALIA,	)	
	)	
Defendant/Counter-Plaintiffs,	)	
	)	
vs.	)	
	)	
NIPPON SHINYAKU CO., LTD., and	)	
NS PHARMA, INC.,	)	
	)	
Plaintiff/Counter-Defendants.	)	
<hr/>		

HIGHLY CONFIDENTIAL TRANSCRIPT

VIDEO DEPOSITION OF STEVEN F. DOWDY, PHD

NOVEMBER 8, 2023

SAN DIEGO, CALIFORNIA

Job No. 1123

Pages 1 - 252

Reported by

Cynthia J. Vega, RMR, RDR, CSR 6640, CCRR 95

<p>1 APPEARANCES Page 2</p> <p>2</p> <p>3 On behalf of the Plaintiff and Counter-Defendants:</p> <p>4 MORGAN, LEWIS &amp; BOCKIUS LLP</p> <p>5 By: Amanda S. Williamson, Esq.</p> <p>6 Guylaine Hache, Esq.</p> <p>7 110 North Wacker Drive</p> <p>8 Chicago, Illinois 60606</p> <p>9 (312) 324-1450</p> <p>10 amanda.williamson@morganlewis.com</p> <p>11 guylaine.hache@morganlewis.com</p> <p>12</p> <p>13 On behalf of the Defendant and Counter-Plaintiffs:</p> <p>14 FINNEGAN, HENDERSON, FARABOW, GARRETT &amp; DUNNER, LLP</p> <p>15 By: William B. Raich, Esq.</p> <p>16 Yoonjin Lee, Esq.</p> <p>17 901 New York Avenue, NW</p> <p>18 Washington, DC 20001</p> <p>19 (202) 408-4000</p> <p>20 william.raich@finnegan.com</p> <p>21 yoonjin.lee@finnegan.com</p> <p>22</p> <p>23 The Videographer:</p> <p>24 Jaylen Bell</p> <p>25 * * * * *</p>	<p>1 INDEX Page 4</p> <p>2 WITNESS</p> <p>3 Steven F. Dowdy, PhD</p> <p>4</p> <p>5 EXAMINATION PAGE</p> <p>6 By Ms. Williamson 7</p> <p>7 By Mr. Raich 248</p> <p>8</p> <p>9</p> <p>10</p> <p>11 EXHIBITS</p> <p>12 EXHIBIT DESCRIPTION PAGE</p> <p>13 Exhibit 1 Opening Expert Report of Steven 13</p> <p>14 F. Dowdy, PhD</p> <p>15 Exhibit 2 Rebuttal Expert Report of Steven 13</p> <p>16 F. Dowdy, PhD</p> <p>17 Exhibit 3 Reply Expert Report of Steven F. 13</p> <p>18 Dowdy, PhD</p> <p>19 Exhibit 4 Expert Report of Dr. Michelle L. 52</p> <p>20 Hastings Regarding Invalidity of</p> <p>21 the UWA Patents</p> <p>22 Exhibit 5 United States Patent Number 61</p> <p>23 9,994,851</p> <p>24 Exhibit 6 Expert Rebuttal Report of 88</p> <p>25 Dr. Matthew J.A. Wood</p>
<p>1 The video deposition of Steven F. Dowdy, Page 3</p> <p>2 PhD, a Witness herein, taken on behalf of Plaintiff</p> <p>3 and Counter-Defendants, on Wednesday, November 8,</p> <p>4 2023, before Cynthia J. Vega, CSR 6640, beginning at</p> <p>5 the hour of 8:13 a.m., at 12670 High Bluff Drive, in</p> <p>6 the City of San Diego, County of San Diego, State of</p> <p>7 California.</p> <p>8</p> <p>9</p> <p>10</p> <p>11</p> <p>12</p> <p>13</p> <p>14</p> <p>15</p> <p>16</p> <p>17</p> <p>18</p> <p>19</p> <p>20</p> <p>21</p> <p>22</p> <p>23</p> <p>24</p> <p>25</p>	<p>1 EXHIBITS Page 5</p> <p>2 EXHIBIT DESCRIPTION PAGE</p> <p>3 Exhibit 7 Sarepta Therapeutics Research 101</p> <p>4 Report</p> <p>5 Exhibit 8 United States Patent Application 108</p> <p>6 Publication US 2010/0168212 A1</p> <p>7 Exhibit 9 Responsive Expert Report of 110</p> <p>8 Dr. Michelle L. Hastings</p> <p>9 Regarding the Validity of the NS</p> <p>10 Patents</p> <p>11 Exhibit 10 Patent Application Number 149</p> <p>12 14/615,504, Patent Number</p> <p>13 9,708,361</p> <p>14 Exhibit 11 International Publication Number 168</p> <p>15 WO 2010/048586 A1</p> <p>16 Exhibit 12 Reply Expert Report of 203</p> <p>17 Dr. Michelle L. Hastings</p> <p>18 Regarding the Invalidity of the</p> <p>19 UWA Patents</p> <p>20 Exhibit 13 Ms. Adams' Lab Notebook Number 1 229</p> <p>21</p> <p>22</p> <p>23</p> <p>24</p> <p>25</p>

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1 SAN DIEGO, CALIFORNIA  
2 WEDNESDAY, NOVEMBER 8, 2023, 8:13 A.M.  
3  
4 THE VIDEOGRAPHER: Good morning. We are on  
5 the video record on November 8, 2023, and the time  
6 is 8:13 a.m.  
7 My name is Jaylen Bell. I am the legal  
8 videographer and notary public. And the court  
9 reporter today is Cindy Vega.  
10 This is the beginning of media unit 1 in  
11 the deposition of Dr. Steven F. Dowdy in the matter  
12 of Nippon Shinyaku versus Sarepta Therapeutics. The  
13 case number is 21-1015.  
14 We are located at 12670 High Bluff Drive,  
15 San Diego, California 92130.  
16 And, Counsel, would you please identify  
17 yourselves for the record, starting with the  
18 noticing attorney, please.  
19 MS. WILLIAMSON: Good morning. This is  
20 Amanda Williamson and Guylaine Hache from Morgan  
21 Lewis on behalf of Nippon Shinyaku and NS Pharma.  
22 MR. RAICH: Bill Raich of Finnegan for  
23 Sarepta, the University of Western Australia, and  
24 the witness. With me today is my colleague Yoonjin  
25 Lee, also of Finnegan.

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1 THE VIDEOGRAPHER: Thank you.  
2 The videographer and court reporter are  
3 both here representing EcoScribe.  
4 And the court reporter may now swear in the  
5 witness.  
6  
7 STEVEN F. DOWDY, PhD,  
8 Witness herein, being first duly sworn, testifies as  
9 follows:  
10  
11 EXAMINATION  
12 BY MS. WILLIAMSON:  
13 Q. Good morning, Dr. Dowdy. Thank you for  
14 sitting for this deposition today. We appreciate  
15 your time. We recognize that you already spent  
16 significant time in preparing your reports.  
17 Have you been deposed before?  
18 A. Once.  
19 Q. And when was that?  
20 A. That was approximately six years ago. I  
21 was a witness for a tax issue with a biotech  
22 company.  
23 Q. Were you a witness in an expert capacity or  
24 a fact capacity?  
25 A. As a fact capacity.

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1 Q. So is this your first expert deposition?  
2 A. Yes, it is.  
3 Q. So I'm going to go over some basic ground  
4 rules. I'm sure Mr. Raich has already gone over  
5 them with you, but I'll just give a quick refresher.  
6 Just want to make sure you understand  
7 you're testifying under oath today as if you were  
8 testifying in court. Is that your understanding?  
9 A. Yes.  
10 Q. And that my questions and your answers will  
11 be recorded by the court reporter. Is that your  
12 understanding?  
13 A. I understand.  
14 Q. And so I will do my best to let you finish  
15 your answers before I start my next question and I  
16 hope that you can do the same so the court reporter  
17 can accurately record the testimony. Is that fair?  
18 A. I can.  
19 Q. And also I'd ask that you give a verbal  
20 response to my questions as she cannot record  
21 nodding or hand gestures as you would in normal  
22 conversation. Is that all right?  
23 A. I understand.  
24 Q. And please let me know if you don't  
25 understand my question, but if you answer, I will

Page 9

1 assume that you have understood the question as  
2 posed. Is that fair?  
3 A. Yes.  
4 Q. And if you want to take a break at any  
5 time, please let me know, and we will take a break  
6 at the next opportunity. I may ask you to finish a  
7 brief line of questioning or fully answer the  
8 question that is pending at the time. Is that all  
9 right?  
10 A. Yes. I understand.  
11 Q. And do you understand that you're required  
12 to answer my question to the best of your ability?  
13 A. Yes, I do.  
14 Q. And that your counsel may pose objections  
15 from time to time?  
16 A. Yes. They may.  
17 Q. And that unless they specifically instruct  
18 you not to answer the question, you'll still be  
19 required to answer my question even though they have  
20 objected. Is that your understanding?  
21 A. That is my understanding.  
22 Q. Is there any reason you cannot testify  
23 fully and truthfully today?  
24 A. No.  
25 Q. Are you being compensated for your time



<p style="text-align: right;">Page 14</p> <p>1 A. I do.</p> <p>2 Q. And you've identified here both the claim</p> <p>3 terms and the Court's construction for terms that</p> <p>4 were construed by this Court earlier -- by the Court</p> <p>5 earlier in this case; is that correct?</p> <p>6 A. That is correct.</p> <p>7 Q. And so you're aware that those terms</p> <p>8 include a base sequence, a target region, and the</p> <p>9 target region is within annealing site H53A, 23 to</p> <p>10 47, and annealing site H53A, 39 to 69?</p> <p>11 A. Yes, I am.</p> <p>12 Q. In paragraph 30 you further state that</p> <p>13 you've "applied the construction provided by the</p> <p>14 Court. For all other terms that are not construed</p> <p>15 by the Court, I have applied the plain meaning of</p> <p>16 those terms as understood by a POSA, consistent with</p> <p>17 the disclosures of the Wilton patents and the</p> <p>18 NS patents as of their effective filing dates."</p> <p>19 Do you see that?</p> <p>20 A. Yes, I do.</p> <p>21 Q. And is that -- do you understand -- do you</p> <p>22 understand that in your current opinions that where</p> <p>23 the Court has presented a construction, have you</p> <p>24 complied with the Court's order and applied that</p> <p>25 construction?</p>	<p style="text-align: right;">Page 16</p> <p>1 oligonucleotide of the UWA claims does not need to</p> <p>2 be 100 percent complementary to the dystrophin</p> <p>3 pre-mRNA; is that correct?</p> <p>4 A. The term antisense oligonucleotide as used</p> <p>5 in the field by a POSA would be ideally 100 percent</p> <p>6 complementary; however, you can tolerate one or</p> <p>7 perhaps two mismatches.</p> <p>8 Q. So you would agree that your proposed</p> <p>9 construction does not require that the antisense</p> <p>10 oligonucleotides of the claim be 100 percent</p> <p>11 complementary to the -- does not need to be</p> <p>12 100 percent complementary to the dystrophin pre-mRNA</p> <p>13 as you've explained?</p> <p>14 A. It needs to be 100 percent or nearly</p> <p>15 100 percent identical to the target region.</p> <p>16 Q. And in your construction, you used the</p> <p>17 phrase "highly complementary." Do you see that?</p> <p>18 A. Yes.</p> <p>19 Q. What does that mean to you?</p> <p>20 A. Nearly complementary.</p> <p>21 Q. What does nearly complementary mean to you?</p> <p>22 A. Very close to 100 percent.</p> <p>23 Q. And what is very close to 100 percent?</p> <p>24 A. So an antisense oligonucleotide, depending</p> <p>25 on the length and the type of chemistry, could</p>
<p style="text-align: right;">Page 15</p> <p>1 A. Yes, I have.</p> <p>2 Q. Do you understand that Sarepta did not</p> <p>3 identify the term antisense oligonucleotide as a</p> <p>4 disputed term in need of construction?</p> <p>5 A. Yes, I do.</p> <p>6 Q. And do you understand that the Court</p> <p>7 construed the term target region and adopted a</p> <p>8 construction proposed by Sarepta as a segment of</p> <p>9 pre-mRNA without further limitation?</p> <p>10 A. Yes, I am.</p> <p>11 Q. Let's turn to Exhibit 2, please. If you</p> <p>12 can turn please to Exhibit 2, paragraph 37.</p> <p>13 A. I have it in front of me.</p> <p>14 Q. In paragraph 37 you're offering an</p> <p>15 additional construction for the term antisense</p> <p>16 oligonucleotide as it appears in the UWA patent</p> <p>17 claims; is that correct?</p> <p>18 A. Yes.</p> <p>19 Q. And your construction for antisense</p> <p>20 oligonucleotide is that the antisense</p> <p>21 oligonucleotide is highly complementary, if not</p> <p>22 100 percent complementary, to their target regions;</p> <p>23 is that correct?</p> <p>24 A. That is correct.</p> <p>25 Q. So in your opinion, the antisense</p>	<p style="text-align: right;">Page 17</p> <p>1 tolerate one or perhaps two mismatches, which would</p> <p>2 be highly complementary or nearly 100 percent</p> <p>3 complementary to the target region. And if you</p> <p>4 start to introduce more mismatches than that, then</p> <p>5 it drops off into exceedingly low odds that it would</p> <p>6 hybridize to the target region.</p> <p>7 Q. You said mismatches, that one to two</p> <p>8 mismatches would be permissible. What about</p> <p>9 insertions, are insertions permissible?</p> <p>10 A. Similarly -- I was going to insert that --</p> <p>11 I just didn't -- into my answer.</p> <p>12 Yes. Similarly you could have one or</p> <p>13 perhaps two insertions into the sequence depending</p> <p>14 on the length, depending on the sequence.</p> <p>15 Q. And what about deletions, could you also</p> <p>16 have deletions?</p> <p>17 A. Deletions become more problematic because</p> <p>18 they shorten the length of the two opposite sides of</p> <p>19 the deletion. But if you're asking could you</p> <p>20 tolerate one deletion or a nearly identical ASO</p> <p>21 sequence with the deletion, then the answer would be</p> <p>22 yes.</p> <p>23 Q. And why does the shortening of length</p> <p>24 matter when you're talking about the deletions?</p> <p>25 MR. RAICH: I'll object to the form of the</p>

<p style="text-align: right;">Page 22</p> <p>1 BY MS. WILLIAMSON: 2 Q. And how do you draw the line for an 3 antisense oligonucleotide to say this has too much 4 off-site binding so it's no longer an antisense 5 oligonucleotide? How do I determine that? 6 MR. RAICH: Objection. Incomplete 7 hypothetical. 8 THE WITNESS: It's going to vary between 9 target sequence, length of oligo, and type of 10 chemistry. 11 BY MS. WILLIAMSON: 12 Q. So is it fair to say that for longer 13 oligonucleotides, you would likely be able to 14 tolerate a larger number of mismatches? 15 A. That would be potentially you could 16 tolerate one or two, but you also, by having a 17 longer oligonucleotide, you increase the potential 18 for off target. 19 Q. So there may not be a direct correlation 20 between length and the number of mismatches that 21 could be included? 22 A. This is a very large world of hypothetical 23 antisense oligonucleotides, so it would involve all 24 of the parameters I just outlined. 25 Q. And so is it fair to say that you would</p>	<p style="text-align: right;">Page 24</p> <p>1 looking at the sequence? Could you determine 2 whether it would have the potential for more than 3 permissible off-target binding? 4 MR. RAICH: Objection to the form of the 5 question. 6 THE WITNESS: Yeah. I don't actually 7 understand the question. 8 BY MS. WILLIAMSON: 9 Q. So if I were to pick, let's see, from 10 table 1 of your report on page 29, antisense 11 oligonucleotide targeting, let's say, positions 32 12 to 52, how could I determine how many or if any 13 mismatches would be permissible such that it didn't 14 cause too much off-target binding? 15 A. Number one, 32 to 52 is in the target 16 region, so as all the other ASOs that land within 17 the target region, that would induce exon skipping. 18 And the number of mismatches that you could 19 tolerate, that's a 30-mer -- I'm sorry -- that's a 20 21-mer. That would potentially tolerate one, again, 21 depending on the location of that. And as you get 22 to two or three or four, then it drops off 23 exceedingly rapidly. 24 Q. And so with respect to the plus 32 to 25 plus 52, can you predict whether it can tolerate two</p>
<p style="text-align: right;">Page 23</p> <p>1 have to test the oligonucleotides to determine 2 whether there was more than allowable off-target 3 binding? 4 MR. RAICH: Objection. Incomplete 5 hypothetical. 6 THE WITNESS: Again, it depends on the 7 target region that you're going after. That is 8 going to vary with target region and with chemistry. 9 BY MS. WILLIAMSON: 10 Q. And when you say -- I guess speaking here 11 today, I would confine our -- at least for purposes 12 of this discussion, our target region to somewhere 13 on the exon 53 pre-mRNA. Would that be fair? 14 A. Sure. And what's the question? I'm sorry. 15 Q. And so the question is considering -- does 16 the fact that we are looking at the exon 53 pre-mRNA 17 impact your analysis as to whether -- as to whether 18 or how much off-target binding there will be? 19 A. Again, it depends on the specific sequence 20 within exon 53 and the type of chemistry of the 21 oligonucleotide. 22 Q. Okay. And going back to the second part of 23 my earlier question, setting aside the empirical 24 testing, how would you make a determination, looking 25 at a sequence that does target exon 53, just by</p>	<p style="text-align: right;">Page 25</p> <p>1 mismatches and still bind sufficiently in your -- 2 under your definition to be an antisense 3 oligonucleotide within the claims? 4 A. No, I cannot. 5 Q. And what about with three or four 6 mismatches? 7 A. I guess are you asking me how I would know 8 that or how I would experimentally determine that? 9 Q. So I guess the first question: Can you 10 know that just by looking at the sequence and by me 11 proposing to you a number of mismatches? 12 A. No, you can't know that. 13 Q. So you would need to determine empirically? 14 A. Yes. But you would have the highest 15 probability with 100 percent identical, and then a 16 single mismatch would have a very high probability. 17 And then you would start dropping off from there as 18 you introduce more mismatches. And this has already 19 been performed by Mann, et al. 20 Q. Mann has tested all the versions of the 21 antisense oligonucleotides listed in table 1 with 22 mismatches? 23 A. No. My reference to Mann was that it 24 has -- they've looked at increasing the number of 25 mismatches and where you drop off activity and</p>

<p style="text-align: right;">Page 66</p> <p>1 BY MS. WILLIAMSON:  2 Q. Yes.  3 A. Yes.  4 Q. And so in your opinion, what claim  5 limitations does R2 fail to meet?  6 A. The same as R1, that it's not consecutive  7 bases.  8 Q. Would you have predicted that R2 would  9 induce exon skipping?  10 A. I would have predicted that it would be a  11 very low probability, similar to the low probability  12 of the 12 nucleotide, antisense oligonucleotide to  13 exon 19. But because it's outside of the hot spot,  14 it doesn't exclude that it can't induce exon  15 skipping. The specification nor the claims say that  16 only exon skipping can occur within the 23 to 69  17 nucleotides, not outside of that.  18 Q. Aren't a significant number of the  19 antisense oligonucleotides that you contemplate in  20 the genus that encompasses the '590 and '827 patents  21 as reported in paragraph 58 of your rebuttal report,  22 aren't those also outside of the 23 to 69 purported  23 hot spot?  24 A. These are consecutive bases that are  25 outside with the 12 or more nucleotide overlap of</p>	<p style="text-align: right;">Page 68</p> <p>1 chance that they will not induce exon skipping?  2 MR. RAICH: I'll object to the form of the  3 question.  4 THE WITNESS: Yeah. That's a pretty strong  5 statement. I would say that what's consistent with  6 all the data in the Wilton patent and subsequently  7 that I have been aware of that I read, that I've  8 seen, that you have the highest probability for  9 success within the hot spot as defined by the  10 claims. And as you go out of the hot spot, the  11 probability for success drops off.  12 So that's not to say that if you're -- the  13 Wilton patent does not say that if you're outside  14 the hot spot, that you will not induce exon  15 skipping. And there's examples from other work that  16 have antisense oligonucleotides in exon 53 that are  17 outside the hot spot that cause -- that have been  18 reported to cause skipping.  19 BY MS. WILLIAMSON:  20 Q. But it's at least somewhat less probability  21 outside the hot spot than it would be inside the hot  22 spot in your opinion?  23 A. No. I think you're slightly twisting my  24 words, perhaps not intentionally. That within the  25 hot spot you have an extremely high probability that</p>
<p style="text-align: right;">Page 67</p> <p>1 the target region. And so I think the operative  2 word here is that they have the potential that they  3 could induce exon skipping because they overlap  4 substantially with the hot spot.  5 Q. But they're not completely within that hot  6 spot, you would agree?  7 A. They have sufficient overlap with the hot  8 spot as defined in the claims.  9 Q. And for you that sufficient overlap is  10 12 base pairs of SEQ ID 195?  11 A. As written, yes.  12 Q. And so just as an example looking at the  13 table that precedes the text of paragraph 58,  14 starting with plus 14 plus 34 and going to plus 22  15 to plus 42, those antisense oligomers include  16 regions -- bases or regions that are outside the  17 purported hot spot at 23 to 69; is that right?  18 A. They do.  19 Q. And in your opinion, what effect does that  20 have on their -- or your predictive ability or your  21 ability to predict what kind of -- whether or not  22 they -- strike that. We're too far down the road.  23 Would you -- would you agree or is it your  24 opinion that the further outside of the hot spot the  25 antisense oligonucleotides shift, the greater the</p>	<p style="text-align: right;">Page 69</p> <p>1 you will induce exon skipping. It says nothing  2 about the probability on the outside of the hot  3 spot.  4 Q. What about the probability for those  5 antisense oligonucleotides that are outside the hot  6 spot but still within the 12 base requirement of the  7 SEQ ID 195?  8 A. If they fulfill the claims, then there  9 would be a very high probability that they would  10 induce exon skipping.  11 Q. Is it a greater probability or a lesser  12 probability than those fully inside the hot spot or  13 you can't determine?  14 A. I don't have -- no, I cannot determine. If  15 it fulfills the claims and it's within the hot spot  16 per the claims, then every one of those, including  17 the ones in the CERI report that are actual  18 antisense oligonucleotides, induce exon skipping.  19 Excuse me.  20 Q. And is that expectation based on all of the  21 real world evidence that you cite in your report,  22 that everything in the hot spot will work?  23 MR. RAICH: Objection. Foundation.  24 THE WITNESS: It's premised on the Wilton  25 2005 patent and the specification there. And then</p>

<p style="text-align: right;">Page 70</p> <p>1 it's proven by all the real world data subsequently. 2 BY MS. WILLIAMSON: 3 Q. What if the real world data had refuted the 4 hot spot, would your opinion be different as to 5 whether the hot spot existed in the first place? 6 A. Number one, that's not the case, so I 7 can't -- as a scientist, I can't answer that 8 question because I factually know that every oligo 9 that is in the hot spot induces exon 53 skipping. 10 Q. Dr. Dowdy, let's turn to Exhibit 1, which 11 is your opening report. You can set aside 12 Dr. Hastings' report, but I would keep your rebuttal 13 report handy because there is some back and forth 14 that's going to be required. 15 A. Okay. 16 Q. In paragraph 99 in the second paragraph -- 17 A. I'm sorry. You'll have to give me a second 18 to get there. 19 Q. I'm sorry. 20 A. Okay. I'm on paragraph 99 of my expert 21 report. 22 Q. If you look in the middle of the paragraph 23 after the first sentence, you state that "It is 24 readily apparent that some of the ASOs that were 25 reported to induce exon 53 skipping are clustered</p>	<p style="text-align: right;">Page 72</p> <p>1 Although he never uses the word hot spot in his 2 patent, does he? 3 A. Correct. That's a molecular biology ASO 4 RNAi slang word that encompasses the area where the 5 oligonucleotides have high activity. 6 Q. Had you used that term in the course of 7 your work before starting this litigation? 8 A. Yeah. We would say that where -- when 9 antisense oligonucleotides don't -- they can bind 10 throughout the RNA, but binding to different regions 11 has increased or decreased activity depending on 12 primarily that structure. So you would say that's a 13 hot spot because you can have a series of them in 14 that area. 15 Q. And you've specifically used the term hot 16 spot to describe that area in your work? 17 A. It is a generalized slang. 18 Q. Have you yourself used it in your work to 19 describe an area of active binding? 20 A. I'm sure that I have. I could not give you 21 a precise date and time when I used that word. It's 22 again a generic slang in molecular biology. 23 Q. Okay. For Wilton's purported hot spot, is 24 it fair to say that you have -- or he has drawn the 25 bounds of it -- strike that.</p>
<p style="text-align: right;">Page 71</p> <p>1 between the 23rd to the 63rd nucleotides of human 2 exon 53, starting from H53A plus 23 plus 47 which 3 showed very faint skipping, and ending with H53A 4 plus 23 plus 69 which showed strong skipping." 5 Do you see that? 6 A. Yeah. Just to correct, it is plus 39 to 7 plus 69. 8 Q. Excuse me. Yes. That is correct. 9 A. Yes, I do see that sentence. 10 Q. If you could also turn to page -- actually 11 it will be easier because we already have it out. 12 If you'd go to Exhibit Number 5 and turn to 13 table 39, please. 14 A. Can you tell me what page that's on? 15 Q. Yes. I'll get you the -- it is the page 16 number ending in 717, column 65. 17 A. Yes, I have it open. 18 Q. And so is it fair to say that your 19 identification of the hot spot is based on the very 20 faint skipping shown by 23 to 47 or the -- strike 21 that. 22 The bounds of the hot spot that you purport 23 to identify -- 24 A. I didn't purport to identify. 25 Q. Or that Wilton purports to identify.</p>	<p style="text-align: right;">Page 73</p> <p>1 Is it fair to say that for the hot spot 2 that Wilton -- that you claim Wilton has identified, 3 you rely on plus 23 plus 47's very faint skipping to 4 define the lower boundary of the hot spot? 5 A. The nucleotide lower boundary, yes, the 6 5 prime end of the hot spot. 7 Q. And for the other boundary, or I guess 8 we'll call it the 3 prime boundary, you've relied on 9 the plus 39 plus 69 oligonucleotides strong 10 skipping? 11 A. That which is confirmed with the faint 12 skipping with sequence ID number 191, which is 45 to 13 69. 14 Q. And would you agree with me that plus -- 15 that in table 39 of Exhibit 5, the plus 14 minus 7 16 oligonucleotide also shows very faint skipping? 17 A. Yes, as reported in the table. 18 Q. And would you agree with me that the plus 9 19 minus 18 oligonucleotide is reported in table 39 as 20 showing faint skipping? 21 A. Plus 9 to minus 19, yes. 22 Q. And would you agree with me that the 23 plus 14 minus 7 and plus 9 minus 18 oligonucleotides 24 overlap in base sequence? 25 MR. RAICH: Objection. Foundation.</p>



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1 BY MS. WILLIAMSON:  
2 Q. I guess a lot of times in the antibody  
3 space we hear antibodies described as being  
4 90 percent over --  
5 A. Yeah.  
6 Q. -- 90 percent overlapping or having  
7 90 percent of the sequence. Is there any similar  
8 thinking with respect to antisense oligonucleotides?  
9 A. No. It's actually --  
10 MR. RAICH: Object to the form of the  
11 question. Incomplete hypothetical.  
12 THE WITNESS: It's -- in the world of RNA  
13 therapeutics, you have the sequence of the target  
14 region, and so it's substantially different than an  
15 antibody.  
16 BY MS. WILLIAMSON:  
17 Q. And so you wouldn't base your assessment  
18 of -- your prediction on, let's say, exon skipping  
19 based on percent commonality with another AO?  
20 MR. RAICH: Objection. Incomplete  
21 hypothetical.  
22 THE WITNESS: It would be within, again,  
23 the hot spot.  
24 BY MS. WILLIAMSON:  
25 Q. So you would base it on location rather

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1 than commonality?  
2 A. You're conflating two things together  
3 because location is commonality because we have a  
4 linear sequence. So whereas an antibody, you're  
5 coming with a whole collection of random antibodies  
6 similar to small molecules that you're screening to  
7 bind the target. Here we already know the target.  
8 It's linear. And so the sequence, the target region  
9 is the commonality with all of the ASOs. So it's a  
10 simpler system than an antibody.  
11 Q. You would agree that the -- is there a  
12 percentage of overlap that you would look towards, a  
13 linear overlap?  
14 A. Again, it's --  
15 MR. RAICH: Same objections as before.  
16 Incomplete hypothetical. Form.  
17 THE WITNESS: So I can't answer that  
18 question definitively, but again, it's the hot spot  
19 and the claims that cover the oligos. All of the  
20 oligos that are in the hot spot that fulfill all the  
21 criterion of the claims that we're aware of that I  
22 have reviewed are, in fact, induce exon 53 skipping.  
23 And that goes to your question of predictability.  
24 BY MS. WILLIAMSON:  
25 Q. And just to be clear, that's -- when you

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1 say that everyone that you've reviewed, you've only  
2 reviewed the portion of the genus that is  
3 100 percent -- you only reviewed AOs that would be  
4 100 percent complementary to the pre-mRNA; correct?  
5 You've only reviewed skipping data?  
6 MR. RAICH: Object to the form of the  
7 question.  
8 THE WITNESS: I believe so.  
9 BY MS. WILLIAMSON:  
10 Q. You would agree with me that the UWA  
11 patents disclose only a single sequence that falls  
12 within the scope of the claims; isn't that right?  
13 A. A single member of the genus.  
14 Q. And that's SEQ ID 195; correct?  
15 A. Correct.  
16 Q. You would also agree with me, I think, that  
17 the UWA patents' best performing antisense  
18 oligonucleotide is SEQ ID 193?  
19 A. For activity under those conditions, yes,  
20 it is.  
21 Q. And SEQ ID 193 does not fall within the  
22 claim genus, does it?  
23 A. It overlaps substantially with the claims.  
24 Q. But it does not fall within the genus, does  
25 it?

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1 A. Correct. The genus doesn't say that if you  
2 fall outside of it, that you won't induce skipping.  
3 It says if you fall within the claims, you will  
4 induce skipping.  
5 Q. But you agree with me that there are  
6 portions of the purported hot spot that are outside  
7 the claim genus; correct?  
8 MR. RAICH: Objection to form.  
9 THE WITNESS: I think you might be  
10 conflating the difference between clinical  
11 development and identification of the hot spot. So  
12 the Wilton patents identify the hot spot. That some  
13 of those ASOs have differing activity depending on  
14 the type of cells, et cetera, that are being used  
15 doesn't negate that they're in the hot spot and that  
16 the hot spot was defined and that the 23 to 47 ID  
17 number 195 is a member of that genus.  
18 So it's not a level -- the claims and the  
19 specifications don't tell you you have to have a  
20 therapeutic level of skipping. It identifies the  
21 hot spot and teaches you to explore the hot spot to  
22 find the best clinically viable ASO for a number of  
23 reasons besides just activity: The cost of goods,  
24 sequence, off-target effects, et cetera, safety  
25 profiles, et cetera, et cetera.

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1 BY MS. WILLIAMSON:  
2 Q. I'm more interested in SEQ ID 193 because  
3 that was part of the identification of the hot spot;  
4 correct? It denoted or delineated the upper bound  
5 in plus 69; isn't that true?  
6 A. True. Correct.  
7 Q. And it was the strongest skipper; correct?  
8 A. Off the top of my head, I believe so. If  
9 you'd like to represent that.  
10 Q. I will represent that.  
11 A. Okay.  
12 Q. And it does not fall within the claim  
13 genus? It is not a species?  
14 A. Correct.  
15 Q. And so you were aware of species outside of  
16 the genus that induced exon skipping?  
17 A. Yes. There is also other ASOs from other  
18 groups that are outside of the hot spot that induce  
19 exon skipping. So the hot spot isn't a one or a  
20 zero. It would either fall within it and you induce  
21 exon 53 skipping or you don't if you're outside of  
22 it. It's that it dramatically increases the  
23 probability of success if you are within the hot  
24 spot, which all of the subsequent ASOs, as I've said  
25 multiple times through the day, that fall within the

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1 claims of the Wilton patents also induce exon 53  
2 skipping, and not every ASO that's outside of that  
3 does that. So, of course, there will be other ASOs  
4 that induce skipping. Doesn't exclude that.  
5 Q. And so is it fair to say that the claim  
6 genus does not delineate exclusively an area  
7 function for exon 53 skipping?  
8 A. The specification does not state that exon  
9 skipping will only happen within the confines of the  
10 limitations of claim 1. No, it does not say that.  
11 It says that the probability of success within this  
12 hot spot will be much higher than outside of it, and  
13 it teaches a POSA to then explore the hot spot.  
14 Q. And so going back to your calculations that  
15 include one mismatch for the genus, you'd agree with  
16 me that for the '851 patent you calculated roughly  
17 22,000 species and that for the '590 and '827  
18 patents you calculated roughly 44,000?  
19 A. Correct.  
20 Q. Does that sound fair?  
21 A. Yes.  
22 Q. And is it -- and in your opinion, SEQ  
23 ID 195 is representative of both of -- the entirety  
24 of those claimed genres; is that correct?  
25 A. That is correct.

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1 Q. With respect to your predictability  
2 assessments, you don't dispute that before 2005 the  
3 field of exon skipping was highly unpredictable?  
4 A. Yes. It was.  
5 Q. But in your view the UWA patents resolved  
6 that unpredictability?  
7 MR. RAICH: Objection. Mischaracterizes.  
8 THE WITNESS: For exon 53, the empirical  
9 data derived from Dr. Wood's -- sorry --  
10 Dr. Wilton's laboratory identified the hot spot. So  
11 Dr. Wood's comments of skipping being unpredictable,  
12 that was true until Dr. Wilton's lab found the hot  
13 spot. And then the predictability within the hot  
14 spot went up dramatically versus randomly screening  
15 through ASOs for all the reasons that I cited prior  
16 today.  
17 BY MS. WILLIAMSON:  
18 Q. And when you say "randomly screening  
19 through ASOs," is that for all -- it alleviated that  
20 problem for -- is it your testimony that the hot  
21 spot alleviated that problem for exon 53?  
22 A. Again, it's a hot spot that dramatically  
23 increases the predictability for subsequent studies,  
24 as we discussed earlier, that started from one end  
25 to the other and screened through with

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1 oligonucleotides. And again, they confirmed the  
2 location of the hot spot. That's an independent  
3 analysis that, again, confirms the high  
4 predictability of skipping if you're within the hot  
5 spot and the confines of the claims, the claim  
6 limitations.  
7 Q. But you would agree that the  
8 contemporaneous researchers were screening the  
9 entire exon even after the publication of the Wilton  
10 UWA patent application?  
11 A. Yes. That's a fact. And that's what  
12 scientists do. If in 2005 or 2006 when this was  
13 published and I was doing exon 53 skipping in my  
14 laboratory, I would still have my lab design ASOs  
15 that walked across it. And I would pay attention to  
16 see if we independently identified the hot spot,  
17 which is exactly what happened with multiple  
18 laboratories.  
19 MS. WILLIAMSON: Is this a good time for a  
20 break?  
21 MR. RAICH: Sure.  
22 THE VIDEOGRAPHER: Going off the record at  
23 approximately 3:06 p.m.  
24 (Recess, 3:06 p.m. to 3:27 p.m.)  
25 THE VIDEOGRAPHER: Going back on the record

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1 Q. Do you have an opinion as to whether or not  
2 the claims of the NS patents are novel?  
3 A. I do.  
4 Q. And what is your opinion?  
5 A. They are not novel.  
6 Q. Do you have an opinion as to whether or not  
7 the claims of the NS patents are inventive?  
8 A. I do.  
9 Q. And what is your opinion?  
10 A. They are not.  
11 Q. In view of your opinion, does Prosensa's  
12 failure have a nexus to the claims of the NS  
13 patents?  
14 A. No, it does not.  
15 Q. Given the lack of nexus to the claims of  
16 the NS patents, does Prosensa's failure support the  
17 nonobviousness of the claims of the NS patents?  
18 A. No, it does not.  
19 MR. RAICH: I pass the witness.  
20 MS. WILLIAMSON: No questions.  
21 THE VIDEOGRAPHER: This concludes today's  
22 deposition. We are going off the record at  
23 approximately 5:38 p.m.  
24 (Deposition adjourned at 5:38 p.m.)  
25 \* \* \* \* \*

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1 I declare under the penalty of perjury  
2 under the laws of the State of California, United  
3 States of America, that the foregoing is true and  
4 correct; that I have read my deposition and have  
5 made the necessary corrections, additions or changes  
6 to my answers that I deem necessary.  
7 Dated: \_\_\_\_\_  
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11 \_\_\_\_\_  
12 Steven F. Dowdy, PhD  
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1 CERTIFIED SHORTHAND REPORTER'S CERTIFICATE  
2  
3 I, Cynthia J. Vega, a Certified Shorthand  
4 Reporter for the State of California, do hereby  
5 certify:  
6 That the witness in the foregoing  
7 deposition was by me duly sworn; that the deposition  
8 was then taken before me at the time and place  
9 herein set forth; that the testimony and proceedings  
10 were reported by me stenographically and were  
11 transcribed through computerized transcription under  
12 my direction; and the foregoing is a true and  
13 correct record of the testimony and proceedings  
14 taken at that time.  
15 I further certify that I am not of counsel  
16 or attorney for either or any of the parties in the  
17 foregoing proceeding and caption named or in any way  
18 interested in the outcome of the cause in said  
19 caption.  
20 IN WITNESS WHEREOF, I have subscribed my  
21 name this 13th day of November, 2023.  
22 Reading and Signing was not requested.  
23  
24 *Cindy Vega*  
25 Cynthia J. Vega, CSR No. 6640

**DEPOSITION ERRATA**

Deposition of:

Dr. Steven F. Dowdy

Dated of Deposition:

November 8, 2023

Case:

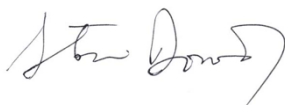
*Nippon Shinyaku Co., Ltd. v. Sarepta Therapeutics, Inc.*

C.A. No. 21-1015 (GBW)

Page	Line	Transcript Text	Correction	Reason
47	24	RNA SAGE	RNaseH	Typographical Error
72	4	RNAi	RNA	Typographical Error
118	22-23	No one used DNA or RNA ASOs prior to 1990.	No one used DNA or RNA ASOs after 1990.	Clarification
139	13-14	Generally you would have an indirect comparison.	Generally you would have a direct comparison.	Clarification
142	1-2	because you get a maximum activity that's nonlinear	because at high concentrations you get a maximum activity that's nonlinear	Clarification
146	17-18	it's maxed out in the linear range of the assay	it's maxed out of the linear range of the assay	Clarification
158	22-23	36 to 60 was superior based on the lower concentration	36 to 60 was inferior based on the lower concentration	Clarification
200	8	work towards 100 hoping to	work towards 100 percent hoping to	Clarification
243	24-25	you can interpret a negative result	you can't interpret a negative result	Typographical Error

I, Steven F. Dowdy, do hereby certify that I have read my deposition transcript dated November 8, 2023; that the changes and corrections to my deposition transcript set forth above are necessary to render the same true and correct; that having made such changes, I hereby subscribe my name to the deposition. I declare under penalty of perjury that the foregoing is true and correct.

Executed this 7 day of December, 2023, at San Diego, California.




---

Dr. Steven F. Dowdy



# Exhibit 2 to NS's MIL No. 1

**THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

NIPPON SHINYAKU CO., LTD.,

Plaintiff,

v.

SAREPTA THERAPEUTICS, INC.,

Defendant.

C.A. No. 21-1015 (GBW)

SAREPTA THERAPEUTICS, INC. and  
THE UNIVERSITY OF WESTERN  
AUSTRALIA,

Defendant/Counter-Plaintiffs,

v.

NIPPON SHINYAKU CO., LTD.  
and NS PHARMA, INC.

Plaintiff/Counter-Defendants.

**REBUTTAL EXPERT REPORT OF STEVEN F. DOWDY, Ph.D.**

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[REDACTED]

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claims, disregards structural features recited in the claims of the Wilton Patents that confer the claimed function of inducing exon 53 skipping, and ignores the representativeness of the disclosed species for the other members of the claimed genera of the Wilton Patents.

31. As appropriately analyzed, the Wilton Patents provide adequate written description support for the claimed inventions. Dr. Wilton and his co-inventors identified a discrete region within human exon 53 that is amenable for exon skipping, i.e., the exon 53 “hot spot.” The claims of the Wilton Patents incorporate this discovery using various structural features, and thereby allow a POSA to visualize the PMOs that potentially fall within the claim scope. The specification provides empirical evidence that these claimed structural features confer the claimed function of inducing exon 53 skipping, and numerous researchers have subsequently confirmed that relationship. The Wilton Patents satisfy the written description requirement.

**1. The Claims of the Wilton Patents Are Narrow in Scope**

**a. Dr. Hastings’ Analysis of Claim Scope Is Premised on a Misreading of the Claims of the Wilton Patents**

32. Dr. Hastings contends that “the genus of claimed antisense oligonucleotides is extremely broad as the genus encompasses upwards of  $10^{14}$  species.” Hastings Rep. ¶50. Dr. Hastings arrives at this number based on the following rationale (*id.*, ¶¶46-48):

(1) “in addition to the ‘base sequence,’ *i.e.*, ‘at least 12 consecutive bases of SEQ ID NO: 195,’ the claimed antisense oligonucleotides can also include additional unspecified bases”;

(2) “[t]hese additional unspecified bases outside of the ‘base sequence’ can be, but do not necessarily have to be complementary to the ‘target region’ set forth in” the claims of the Wilton Patents;



**2. The Wilton Patents Disclose Common Structural Features That Confer the Claimed Function of Inducing Exon 53 Skipping**

**a. A POSA Reading the Wilton Patents Would Have Recognized the Correlation Between the Claimed Structural Features and Claimed Function**

66. All of the asserted claims contain the following common structural features regarding the claimed oligonucleotides: (1) “antisense oligonucleotide”; (2) “20 to 31 bases”; (3) “comprising a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA”; (4) “the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195)”; (5) “in which uracil bases are thymine bases”; and (6) “wherein the antisense oligonucleotide is a morpholino antisense oligonucleotide.” *See* Dowdy Op. Rep. § VII.B. In the case of the ’851 Patent, the claims further require that the target region of the claimed antisense oligonucleotide be present within nucleotides +23 to +69 of human exon 53. *See id.* These structural features identify a relatively small number of candidate ASOs potentially covered by the claims of the Wilton Patents. *See supra* § V.A.1.b.

67. A POSA would have expected that most of these candidate PMOs, if not all, would induce exon 53 skipping because they target the hot spot, or a region overlapping the hot spot, identified by Dr. Wilton and his co-inventors. As discussed in my Opening Expert Report, Dr. Wilton and co-inventors identified a discrete region within exon 53 of the human dystrophin pre-mRNA that is amenable to exon skipping. *See* Dowdy Op. Rep. § V.C.4.b. The identification of the hot spot was based on four ASOs that collectively target nucleotides +23 to +69 of human exon 53 and induced exon skipping. *See* ’851 Patent, Table 39 (reporting exon skipping induced by H53A(+23+47), H53A(+39+62), H53A(+45+69), and H53A(+39+69)); *see also* Wilton Tr. 222:13-223:21 (Dr. Wilton explaining the identification of the hot spot, the beginning of which

expected that the claimed PMOs, which have target regions squarely within or substantially overlapping the hot spot, would similarly induce exon 53 skipping.

72. Real-world evidence that I have reviewed shows that a POSA would have recognized this correlation. As summarized in my Opening Expert Report, a report drafted in 2013-2014, later submitted to the FDA as part of Sarepta's New Drug Application, memorializes the research and development that led to the identification of golodirsen as Sarepta's clinical candidate. *See* SRPT-VYDS-0201524-588 ("Sequence Selection Report"); Dowdy Op. Rep. ¶129. The Sequence Selection Report provides an overview of the exon 53 screens reported in scientific publications and the patent literature, including Wilton PCT '057, which shares a substantively identical disclosure to the Wilton Patents. Sequence Selection Report, SRPT-VYDS-0201529; Dowdy Op. Rep. ¶154. As reproduced below, the report explains that Wilton PCT '057 "identified an effective target region of +23 to +69 relative to the splice acceptor site"—*the same hot spot* that a POSA would have identified from reading the specification of the Wilton Patents.

**Table 1: Summary of Exon 53 Intellectual Property Publications**

Name (coordinates)	Sequence	Publication	SEQ ID NO	Activity*
H53A(+23+47)	CTGAAGGTGTTCTGTACTTCATCC	WO2006/000057	195	Very faint skipping to 50nM RD(3): 16.5%
H53A(+39+69)	CATTCAACTGTTGCCTCCGGTCTGAAGGTG	WO2006/000057	193	Strong skipping to 50nM
H53A(+39+62)	CTGTTGCCTCCGGTCTGAAGGTG	WO2006/000057	192	Faint skipping at 50nM
H53A(+45+69)	CATTCAACTGTTGCCTCCGGTCTG	WO2006/000057	191	Faint skipping at 50nM
NG-08-0578 (+11+40)	TGTTCTGTACTTCATCCCACTGATTCTGA	WO2010/048586	627	RD(3): 4.8%
Ex53.106.30 (+26+55)	CTCCGGTCTGAAGGTGTTCTGTACTTCA	WO2010/048586	629	RD(3): 6.0%
Ex53.115.30 (+35+64)	AACTGTTGCCTCCGGTCTGAAGGTGTTCT	WO2010/048586	632	RD(3): 6.1%
Ex53.118.30 (+38+67)	TTCAACTGTTGCCTCCGGTCTGAAGGTG	WO2010/048586	633	RD(3): 8.1%
H53A(+33+65)	CAACTGTTGCCTCCGGTCTGAAGGTGTTCTTG	WO2011/057350	59	strong skipping to 25nM faint at 2.5
H53A(+33+63)	ACTGTTGCCTCCGGTCTGAAGGTGTTCTTG	WO2011/057350	-	strong skipping to 25nM faint at 5nM
h53A30/1 (+30+59)	TTCCTCCGGTCTGAAGGTGTTCTGTATC	US 2010/0168212	12	52.4%
h53A30/2 (+33+62)	CTGTTGCCTCCGGTCTGAAGGTGTTCTTG	US 2010/0168212	10	87.2%
h53A30/3 (+36+65)	CAACTGTTGCCTCCGGTCTGAAGGTGTTCT	US 2010/0168212	11	80.1%

These studies identified an effective target region at +23 to +69 relative to the splice acceptor site. The four sequences listed above as disclosed in WO2006/000057 (Steve Wilton, University of Western Australia) defined this region and were independently confirmed by work done at Sarepta (WO2010/048586) and at RHUL in Dickson's laboratory (US 2010/0168212). Of the

**Figure 9.** Annotated Excerpt from the Sequence Selection Report (SRPT-VYDS-0201524-588)

73. In sum, the claimed structural features identify a relatively small, finite group of PMOs that potentially fall within the claim scope of the Wilton Patents. Given that the target

these reports, both because it is premised on her misunderstanding of the scope of the claims of the Wilton Patents and because of design flaws with the experiments. *See infra* §§ V.A.4.c.ii-iii.

76. Dr. Hastings also ignores post-filing evidence contrary to her conclusions. For completeness, I reviewed this post-filing evidence, which confirms my opinion that the specification discloses, and the claims require, structural features common to the members of the claimed genera of PMOs, allowing a POSA to visualize or recognize the members of the claimed genera of the Wilton Patents.

77. In my Opening Expert Report, I discussed studies conducted after June 2005 and before August 2011 including: (1) Sazani PCT '586 reporting the work from Drs. Sazani and Kole and (2) publications from Drs. Popplewell and Dickson, including Popplewell 2010. *See* Dowdy Op. Rep. §§ V.C.4.c, X.B.1.a. As shown in Figure 18 in my Opening Expert Report, many of ASOs tested in these studies fall squarely within or overlap the hot spot identified by Dr. Wilton and co-inventors in June 2005. *Id.*, Figure 18.

78. For example, PMO-A, -G, -H, and -I from Drs. Popplewell and Dickson fall within the scope of the claims of the Wilton Patents because they meet the structural requirements of the claims and are reported to induce exon 53 skipping. While an ASO from Drs. Sazani and Kole was tested as a PPMO (PMO conjugated to a peptide), it otherwise meets the structural requirements of the claims and is reported to induce exon skipping. No ASOs meeting the structural requirements of the claims are reported in Popplewell 2010 or Sazani PCT '586 that did not induce exon 53 skipping.

79. The following tables summarize ASOs evaluated in those studies.



ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Sazani (PPMO) SEQ ID NO: 429	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
Popplewell 2010 PMO-A (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+35+59) target region)	Yes (13 consecutive bases)	Yes
Popplewell 2010 PMO-G (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+30+59) target region)	Yes (18 consecutive bases)	Yes
Popplewell 2010 PMO-H (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+33+62) target region)	Yes (15 consecutive bases)	Yes
Popplewell 2010 PMO-I (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+36+65) target region)	Yes (12 consecutive bases)	Yes

**Table 3.** ASOs from Sazani PCT ’586 and Popplewell 2010 and Structural Features of the ’851 Patent Claims

ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Sazani (PPMO) SEQ ID NO: 429	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
Popplewell 2010 PMO-A (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+35+59) target region)	Yes (13 consecutive bases)	Yes
Popplewell 2010 PMO-G (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+30+59) target region)	Yes (18 consecutive bases)	Yes
Popplewell 2010 PMO-H (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+33+62) target region)	Yes (15 consecutive bases)	Yes
Popplewell 2010 PMO-I (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+36+65) target region)	Yes (12 consecutive bases)	Yes

**Table 4.** ASOs from Sazani PCT ’586 and Popplewell 2010 and Structural Features of the ’590 and ’827 Patent Claims

80. Similarly, by August 2011, scientists at NS and NCNP had also confirmed that multiple ASOs meeting all of the structural limitations of the claims of the Wilton Patents induce exon 53 skipping. *See* Dowdy Op. Rep. §§ VI.B.2, VIII.A; '217 Patent, Table 2, Figures 1-8, 18, 19. Dr. Wilton's H53A(+39+69) was used by NS as a positive control for inducing exon skipping in these studies.

81. Notably, among the numerous ASOs reported to induce exon 53 skipping, PMO No. 16 is 100% complementary to the (+23+47) region of human exon 53, i.e., a PMO corresponding to H53A(+23+47) in the Wilton Patents. *See* '217 Patent, Table 2, Figure 18. It was shown to induce exon 53 skipping. Similarly, PMO No. 8 (highlighted in green below) also embodies the structural limits of the claims and was shown to induce exon skipping. This PMO is also known as viltolarsen, the active ingredient of NS's Viltepso<sup>®</sup> (viltolarsen) product. *See* Dowdy Op. Rep. ¶¶138-139, 257; *see id.* § IX.A. Indeed, all of the ASO in the NS Patents that met the structural limitations of the claims of the Wilton Patents induced exon 53 skipping.

82. NS and NCNP scientists had also tested several 2'OMePS ASOs meeting all of the structural imitations of the claims of the Wilton Patents, except that they were not "morpholino" antisense oligonucleotide with thymine bases. *See* '217 Patent, 38:52-54, Figures 9-17, Table 7. Those also are reported to induce exon skipping. *See id.* The following tables summarize the ASOs evaluated by NS and NCNP scientists.

ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
PMO No. 1 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+31+55) target region)	Yes (17 consecutive bases)	Yes
PMO No. 2 (PMO)	Yes (100% complementary)	Yes (22 mer)	Yes (the (+32+53) target region)	Yes (16 consecutive bases)	Yes
PMO No. 3 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
PMO No. 4 (PMO)	Yes (100% complementary)	Yes (22 mer)	Yes (the (+33+54) target region)	Yes (15 consecutive bases)	Yes
PMO No. 5 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+34+58) target region)	Yes (14 consecutive bases)	Yes
PMO No. 7 (PMO)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+36+55) target region)	Yes (12 consecutive bases)	Yes
PMO No. 8 (PMO)	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
PMO No. 9 (PMO)	Yes (100% complementary)	Yes (22 mer)	Yes (the (+36+57) target region)	Yes (12 consecutive bases)	Yes
PMO No. 10 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+33+57) target region)	Yes (15 consecutive bases)	Yes
PMO No. 12 (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+30+59) target region)	Yes (18 consecutive bases)	Yes
PMO No. 13 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
PMO No. 14 (PMO)	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
PMO No. 15 (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+30+59) target region)	Yes (18 consecutive bases)	Yes
PMO No. 16 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
H53_26-50 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+26+50) target region)	Yes (22 consecutive bases)	Yes
H53_31-55 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+31+55) target region)	Yes (17 consecutive bases)	Yes
H53_36-60 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+36+60) target region)	Yes (12 consecutive bases)	Yes
H53_29-53 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+29+53) target region)	Yes (19 consecutive bases)	Yes
H53_30-54	Yes	Yes	Yes	Yes	Yes



ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
(2'OMePS)	(100% complementary)	(25 mer)	(the (+30+54) target region)	(18 consecutive bases)	
H53_32-56 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
H53_33-57 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+33+57) target region)	Yes (15 consecutive bases)	Yes
H53_34-58 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+34+58) target region)	Yes (14 consecutive bases)	Yes
H53_35-59 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+35+59) target region)	Yes (13 consecutive bases)	Yes
H53_32-61 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+32+61) target region)	Yes (16 consecutive bases)	Yes
H53_32-51 (2'OMePS)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+32+51) target region)	Yes (16 consecutive bases)	Yes
H53_35-54 (2'OMePS)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+35+54) target region)	Yes (13 consecutive bases)	Yes

**Table 5.** ASOs from NS’s ’217 Patent and Structural Features of the ’851 Patent Claims

ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
PMO No. 1 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+31+55) target region)	Yes (17 consecutive bases)	Yes
PMO No. 2 (PMO)	Yes (100% complementary)	Yes (22 mer)	Yes (the (+32+53) target region)	Yes (16 consecutive bases)	Yes
PMO No. 3 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
PMO No. 4 (PMO)	Yes (100% complementary)	Yes (22 mer)	Yes (the (+33+54) target region)	Yes (15 consecutive bases)	Yes
PMO No. 5 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+34+58) target region)	Yes (14 consecutive bases)	Yes
PMO No. 7 (PMO)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+36+55) target region)	Yes (12 consecutive bases)	Yes
PMO No. 8 (PMO)	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
PMO No. 9 (PMO)	Yes (100% complementary)	Yes (22 mer)	Yes (the (+36+57) target region)	Yes (12 consecutive bases)	Yes
PMO No. 10 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+33+57) target region)	Yes (15 consecutive bases)	Yes
PMO No. 12 (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+30+59) target region)	Yes (18 consecutive bases)	Yes
PMO No. 13 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
PMO No. 14 (PMO)	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
PMO No. 15 (PMO)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+30+59) target region)	Yes (18 consecutive bases)	Yes
PMO No. 16 (PMO)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
H53_11-35 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+11+35) target region)	Yes (13 consecutive bases)	Yes
H53_16-40 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+16+40) target region)	Yes (18 consecutive bases)	Yes
H53_21-45 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+21+45) target region)	Yes (23 consecutive bases)	Yes
H53_26-50 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+26+50) target region)	Yes (22 consecutive bases)	Yes
H53_31-55	Yes	Yes	Yes	Yes	Yes



ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
(2'OMePS)	(100% complementary)	(25 mer)	(the (+31+55) target region)	(17 consecutive bases)	
H53_36-60 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+36+60) target region)	Yes (12 consecutive bases)	Yes
H53_29-53 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+29+53) target region)	Yes (19 consecutive bases)	Yes
H53_30-54 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+30+54) target region)	Yes (18 consecutive bases)	Yes
H53_32-56 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
H53_33-57 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+33+57) target region)	Yes (15 consecutive bases)	Yes
H53_34-58 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+34+58) target region)	Yes (14 consecutive bases)	Yes
H53_35-59 (2'OMePS)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+35+59) target region)	Yes (13 consecutive bases)	Yes
H53_32-61 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+32+61) target region)	Yes (16 consecutive bases)	Yes
H53_32-51 (2'OMePS)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+32+51) target region)	Yes (16 consecutive bases)	Yes
H53_35-54 (2'OMePS)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+35+54) target region)	Yes (13 consecutive bases)	Yes

**Table 6.** ASOs from NS’s ’217 Patent and Structural Features of the ’590 and ’827 Patent Claims

83. Sarepta's Sequence Selection Report evaluated eight ASOs that share all of the structural features of the claims of the Wilton Patents. All eight ASOs induced exon 53 skipping. *See* Dowdy Op. Rep. § VI.A; Sequence Selection Report, Table 2. Again, all of the ASOs in the Sequence Selection Report that met the structural limitations of the claims of the Wilton Patents induced exon 53 skipping.

84. As discussed above, the Sequence Selection Report states that these eight ASOs were specifically designed to be "internal to Exon 53 and fell within the previously identified effective region (+23+69)." Sequence Selection Report, SRPT-VYDS-0201531. Each of the eight PMOs was reported to induce exon skipping. *See id.*, SRPT-VYDS-0201555 ("There is a clear dose response in the levels of skipping produced by all eight PMOs"). H53A25(+36+60) (highlighted in purple) corresponds to golodirsen, the active ingredient of Sarepta's Vyondys 53<sup>®</sup> (golodirsen) product. *See* Dowdy Op. Rep. § VI.A. Like NS's viltolarsen, Sarepta's golodirsen embodies all of the structural features claimed by the Wilton Patents and is reported to induce exon 53 skipping.



PMO Name	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
H53A31(+33+63)	Yes (100% complementary)	Yes (31 mer)	Yes (the (+33+63) target region)	Yes (15 consecutive bases)	Yes
H53A28(+33+60)	Yes (100% complementary)	Yes (28 mer)	Yes (the (+33+60) target region)	Yes (15 consecutive bases)	Yes
H53A25(+31+55)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+31+55) target region)	Yes (17 consecutive bases)	Yes
H53A30(+36+65)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+36+65) target region)	Yes (12 consecutive bases)	Yes
H53A30(+35+64)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+35+64) target region)	Yes (13 consecutive bases)	Yes
H53A25(+35+59)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+35+59) target region)	Yes (13 consecutive bases)	Yes
H53A25(+36+60)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+36+60) target region)	Yes (12 consecutive bases)	Yes

**Table 7.** ASOs from Sarepta’s Sequence Selection Report and Structural Features of the ’851 Patent Claims



PMO Name	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
H53A31(+33+63)	Yes (100% complementary)	Yes (31 mer)	Yes (the (+33+63) target region)	Yes (15 consecutive bases)	Yes
H53A28(+33+60)	Yes (100% complementary)	Yes (28 mer)	Yes (the (+33+60) target region)	Yes (15 consecutive bases)	Yes
H53A25(+31+55)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+31+55) target region)	Yes (17 consecutive bases)	Yes
H53A25(+22+46)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+22+46) target region)	Yes (24 consecutive bases)	Yes
H53A30(+36+65)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+36+65) target region)	Yes (12 consecutive bases)	Yes
H53A30(+35+64)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+35+64) target region)	Yes (13 consecutive bases)	Yes
H53A25(+35+59)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+35+59) target region)	Yes (13 consecutive bases)	Yes
H53A25(+36+60)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+36+60) target region)	Yes (12 consecutive bases)	Yes

**Table 8.** ASOs from Sarepta’s Sequence Selection Report and Structural Features of the ’590 and ’827 Patent Claims

85. In 2014, two international patent applications from Sarepta were published as WO2014/100714 (“Bestwick PCT ’714”; SRPT-VYDS-0228540-610) and WO2014/153240 (“Bestwick PCT ’240”; SRPT-VYDS-0228611-702), disclosing additional exon 53 targeting ASOs. As summarized below, these documents disclosed additional ASOs that share all of the structural features of the claims of the Wilton Patents and were reported to induce exon skipping. *See* Bestwick PCT ’714, Example 1, Figures 3 & 4; Bestwick PCT ’240, Example 7, Figures 3 & 4. Again, all of the ASOs in these patent publications that met the structural limitations of the claims of the Wilton Patents induced exon 53 skipping.



PMO Name	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Bestwick’714 H53A(+33+60)	Yes (100% complementary)	Yes (28 mer)	Yes (the (+33+60) target region)	Yes (15 consecutive bases)	Yes
Bestwick’714 H53A(+23+47)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
Bestwick’714 H53A(+33+62)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+33+62) target region)	Yes (15 consecutive bases)	Yes
Bestwick’714 H53A(+31+55)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+31+55) target region)	Yes (17 consecutive bases)	Yes
Bestwick’240 H53A(+36+60)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+36+60) target region)	Yes (12 consecutive bases)	Yes
Bestwick’240 H53A(+30+57)	Yes (100% complementary)	Yes (28 mer)	Yes (the (+30+57) target region)	Yes (18 consecutive bases)	Yes
Bestwick’240 H53A(+30+56)	Yes (100% complementary)	Yes (27 mer)	Yes (the (+30+56) target region)	Yes (18 consecutive bases)	Yes
Bestwick’240 H53A(+30+55)	Yes (100% complementary)	Yes (26 mer)	Yes (the (+30+55) target region)	Yes (18 consecutive bases)	Yes
Bestwick’240 H53A(+33+57)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+33+57) target region)	Yes (15 consecutive bases)	Yes

**Table 9.** ASOs from Sarepta’s Patent Publications and Structural Features of the ’851 Patent Claims



PMO Name	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Bestwick’714 H53A(+33+60)	Yes (100% complementary)	Yes (28 mer)	Yes (the (+33+60) target region)	Yes (15 consecutive bases)	Yes
Bestwick’714 H53A(+23+47)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
Bestwick’714 H53A(+33+62)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+33+62) target region)	Yes (15 consecutive bases)	Yes
Bestwick’714 H53A(+31+55)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+31+55) target region)	Yes (17 consecutive bases)	Yes
Bestwick’714 H53A(+22+46)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+22+46) target region)	Yes (24 consecutive bases)	Yes
Bestwick’240 H53A(+36+60)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+36+60) target region)	Yes (12 consecutive bases)	Yes
Bestwick’240 H53A(+30+57)	Yes (100% complementary)	Yes (28 mer)	Yes (the (+30+57) target region)	Yes (18 consecutive bases)	Yes
Bestwick’240 H53A(+30+56)	Yes (100% complementary)	Yes (27 mer)	Yes (the (+30+56) target region)	Yes (18 consecutive bases)	Yes
Bestwick’240 H53A(+30+55)	Yes (100% complementary)	Yes (26 mer)	Yes (the (+30+55) target region)	Yes (18 consecutive bases)	Yes
Bestwick’240 H53A(+33+57)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+33+57) target region)	Yes (15 consecutive bases)	Yes

**Table 10.** ASOs from Sarepta’s Patent Publications and Structural Features of the ’590 and ’827 Patent Claims

86. As I discussed in my Opening Expert Report, I understand that in Europe, NS challenged a patent sharing priority with the Wilton Patents. *See* Dowdy Op. Rep. ¶724. As Dr. Hastings mentioned in her report, I further understand that Sarepta's scientist Dr. Fred Schnell submitted two declarations in this proceeding evaluating ASOs that are 20 to 23 bases in length. *See* Hastings Rep. ¶¶88-89. As summarized below, multiple ASOs sharing all of the structural features of the claims of the Wilton Patents are reported to induce exon skipping.<sup>9</sup> *See* Schnell Decl. 1 (SRPT-VYDS-0228257-67); Schnell Decl. 2 (SRPT-VYDS-0228268-75); *see also* Hastings Rep. ¶¶88-89. As above, all of the ASOs in Dr. Schnell's declarations that met the structural limitations of the claims of the Wilton Patents induced exon 53 skipping.

87. It is also notable that Dr Schnell observed that ASOs that did not contain at least 12 consecutive bases of SEQ ID NO: 195 (and thus would fall outside of the claims of the Wilton Patents) induced a relatively low level of skipping or did not induce exon skipping. *See* Schnell Decl. 2 (e.g., AON8, AON9, AON11, AON17, AON18, AON25, AON26, AON31, AON32).

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<sup>9</sup> Dr. Hastings states: "Several of the antisense oligonucleotides that Dr. Schnell tested fall within the scope of the UWA Patent claims, including antisense oligonucleotides 2, 4, 6, 10, 14, 18, 21, 22, 27 and 28." Hastings Rep. ¶89. The list appears to be incomplete, missing, for example, AON5, 12, 13, and 19. In contrast, AON18, which is included in her list, lacks "at least 12 consecutive bases of" SEQ ID NO: 195 and does not fall within the claim scope of the Wilton Patents.



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Source/ PMO	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Decl. 1 AON2	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
Decl. 1 AON4	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
Decl. 1 AON6	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
Decl. 2 AON2	Yes (100% complementary)	Yes (20 mer)	Yes (the (+32+51) target region)	Yes (16 consecutive bases)	Yes
Decl. 2 AON4	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
Decl. 2 AON5	Yes (100% complementary)	Yes (22 mer)	Yes (the (+23+44) target region)	Yes (22 consecutive bases)	Yes
Decl. 2 AON6	Yes (100% complementary)	Yes (22 mer)	Yes (the (+29+50) target region)	Yes (19 consecutive bases)	Yes
Decl. 2 AON10	Yes (100% complementary)	Yes (23 mer)	Yes (the (+32+54) target region)	Yes (16 consecutive bases)	Yes
Decl. 2 AON12	Yes (100% complementary)	Yes (20 mer)	Yes (the (+23+42) target region)	Yes (20 consecutive bases)	Yes
Decl. 2 AON13	Yes (100% complementary)	Yes (20 mer)	Yes (the (+26+45) target region)	Yes (20 consecutive bases)	Yes
Decl. 2 AON14	Yes (100% complementary)	Yes (20 mer)	Yes (the (+29+48) target region)	Yes (19 consecutive bases)	Yes
Decl. 2 AON19	Yes (100% complementary)	Yes (21 mer)	Yes (the (+23+43) target region)	Yes (21 consecutive bases)	Yes
Decl. 2 AON20	Yes (100% complementary)	Yes (21 mer)	Yes (the (+26+46) target region)	Yes (21 consecutive bases)	Yes
Decl. 2 AON21	Yes (100% complementary)	Yes (21 mer)	Yes (the (+29+49) target region)	Yes (19 consecutive bases)	Yes
Decl. 2 AON22	Yes (100% complementary)	Yes (21 mer)	Yes (the (+32+52) target region)	Yes (16 consecutive bases)	Yes
Decl. 2 AON27	Yes (100% complementary)	Yes (23 mer)	Yes (the (+31+53) target region)	Yes (17 consecutive bases)	Yes
Decl. 2 AON28	Yes (100% complementary)	Yes (23 mer)	Yes (the (+36+58) target region)	Yes (12 consecutive bases)	Yes

**Table 11.** ASOs from Dr. Schnell’s Declarations and Structural Feature of the ’851 Patent Claims

Source/ PMO	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Decl. 1 AON2	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
Decl. 1 AON4	Yes (100% complementary)	Yes (25 mer)	Yes (the (+32+56) target region)	Yes (16 consecutive bases)	Yes
Decl. 1 AON6	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
Decl. 2 AON2	Yes (100% complementary)	Yes (20 mer)	Yes (the (+32+51) target region)	Yes (16 consecutive bases)	Yes
Decl. 2 AON4	Yes (100% complementary)	Yes (21 mer)	Yes (the (+36+56) target region)	Yes (12 consecutive bases)	Yes
Decl. 2 AON5	Yes (100% complementary)	Yes (22 mer)	Yes (the (+23+44) target region)	Yes (22 consecutive bases)	Yes
Decl. 2 AON6	Yes (100% complementary)	Yes (22 mer)	Yes (the (+29+50) target region)	Yes (19 consecutive bases)	Yes
Decl. 2 AON10	Yes (100% complementary)	Yes (23 mer)	Yes (the (+32+54) target region)	Yes (16 consecutive bases)	Yes
Decl. 2 AON12	Yes (100% complementary)	Yes (20 mer)	Yes (the (+23+42) target region)	Yes (20 consecutive bases)	Yes
Decl. 2 AON13	Yes (100% complementary)	Yes (20 mer)	Yes (the (+26+45) target region)	Yes (20 consecutive bases)	Yes
Decl. 2 AON14	Yes (100% complementary)	Yes (20 mer)	Yes (the (+29+48) target region)	Yes (19 consecutive bases)	Yes
Decl. 2 AON19	Yes (100% complementary)	Yes (21 mer)	Yes (the (+23+43) target region)	Yes (21 consecutive bases)	Yes
Decl. 2 AON20	Yes (100% complementary)	Yes (21 mer)	Yes (the (+26+46) target region)	Yes (21 consecutive bases)	Yes
Decl. 2 AON21	Yes (100% complementary)	Yes (21 mer)	Yes (the (+29+49) target region)	Yes (19 consecutive bases)	Yes
Decl. 2 AON22	Yes (100% complementary)	Yes (21 mer)	Yes (the (+32+52) target region)	Yes (16 consecutive bases)	Yes
Decl. 2 AON27	Yes (100% complementary)	Yes (23 mer)	Yes (the (+31+53) target region)	Yes (17 consecutive bases)	Yes
Decl. 2 AON28	Yes (100% complementary)	Yes (23 mer)	Yes (the (+36+58) target region)	Yes (12 consecutive bases)	Yes

**Table 12.** ASOs from Dr. Schnell’s Declarations and Structural Features of the ’590 and ’827 Patent Claims

88. As I discussed in my Opening Expert Report, NS scientist Mr. Watanabe in Europe also submitted declarations in support of NS's challenge to a patent sharing priority with the Wilton Patents. *See* Dowdy Op. Rep. ¶¶726-29. Although omitted from Mr. Watanabe's European declarations, his underlying experiments evaluated two additional PMOs targeting the (+23+43) and (+23+42) regions of human exon 53. *Id.*, ¶¶732-734; *see also* Watanabe Ex. 43, p. 41. As shown below, both PMOs fall squarely within the claim scope of the Wilton Patents and induced exon 53 skipping.



PMO	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
NDK-458 H53_23-43(TEG)	Yes (100% complementary)	Yes (21 mer)	Yes (the (+23+43) target region)	Yes (21 consecutive bases)	Yes
NDK-462 H53_23-42(TEG)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+23+42) target region)	Yes (20 consecutive bases)	Yes

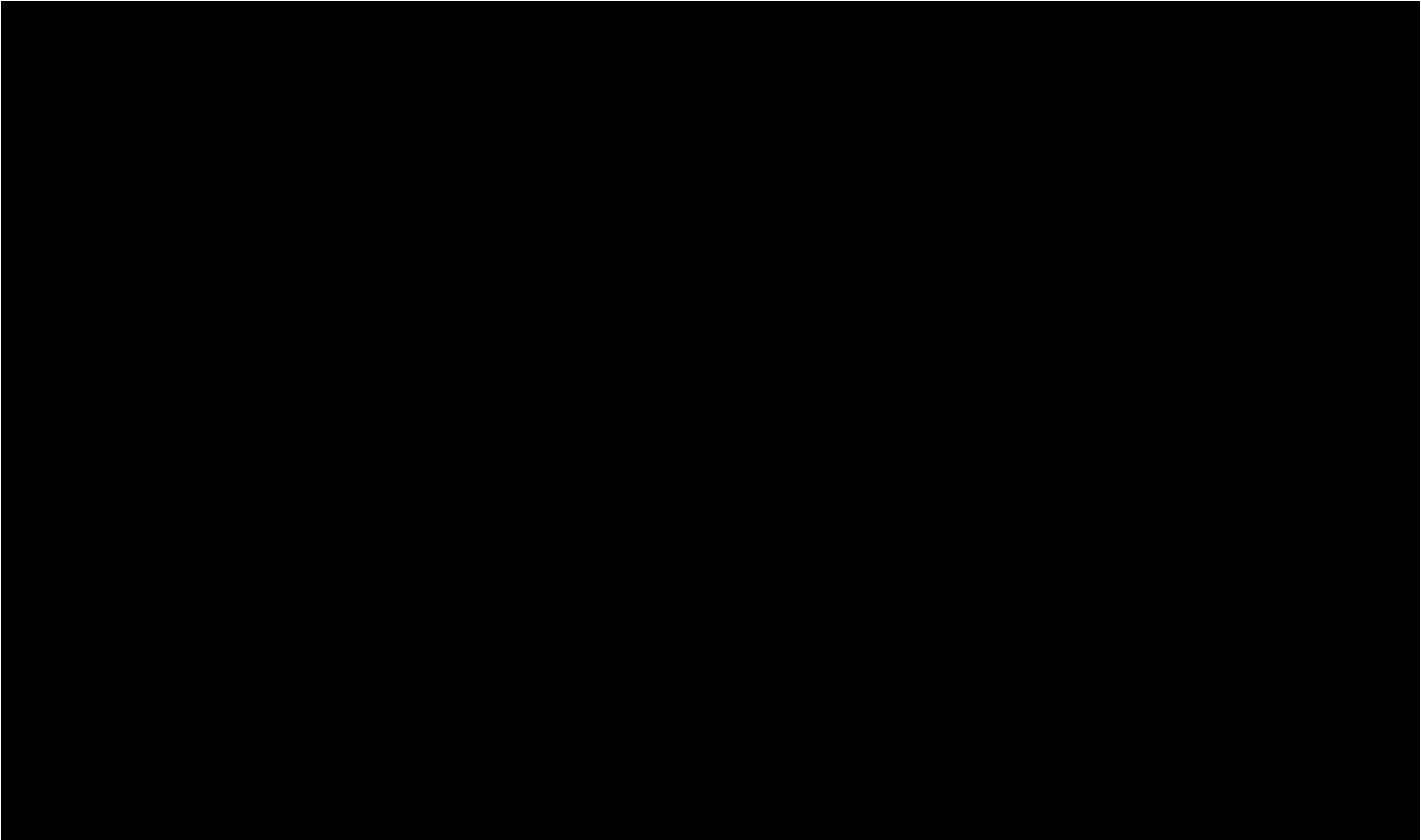
**Table 13.** ASOs from Mr. Watanabe’s Undisclosed Experiment and Structural Features of the ’851 Patent Claims

PMO	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
NDK-458 H53_23-43(TEG)	Yes (100% complementary)	Yes (21 mer)	Yes (the (+23+43) target region)	Yes (21 consecutive bases)	Yes
NDK-462 H53_23-42(TEG)	Yes (100% complementary)	Yes (20 mer)	Yes (the (+23+42) target region)	Yes (20 consecutive bases)	Yes

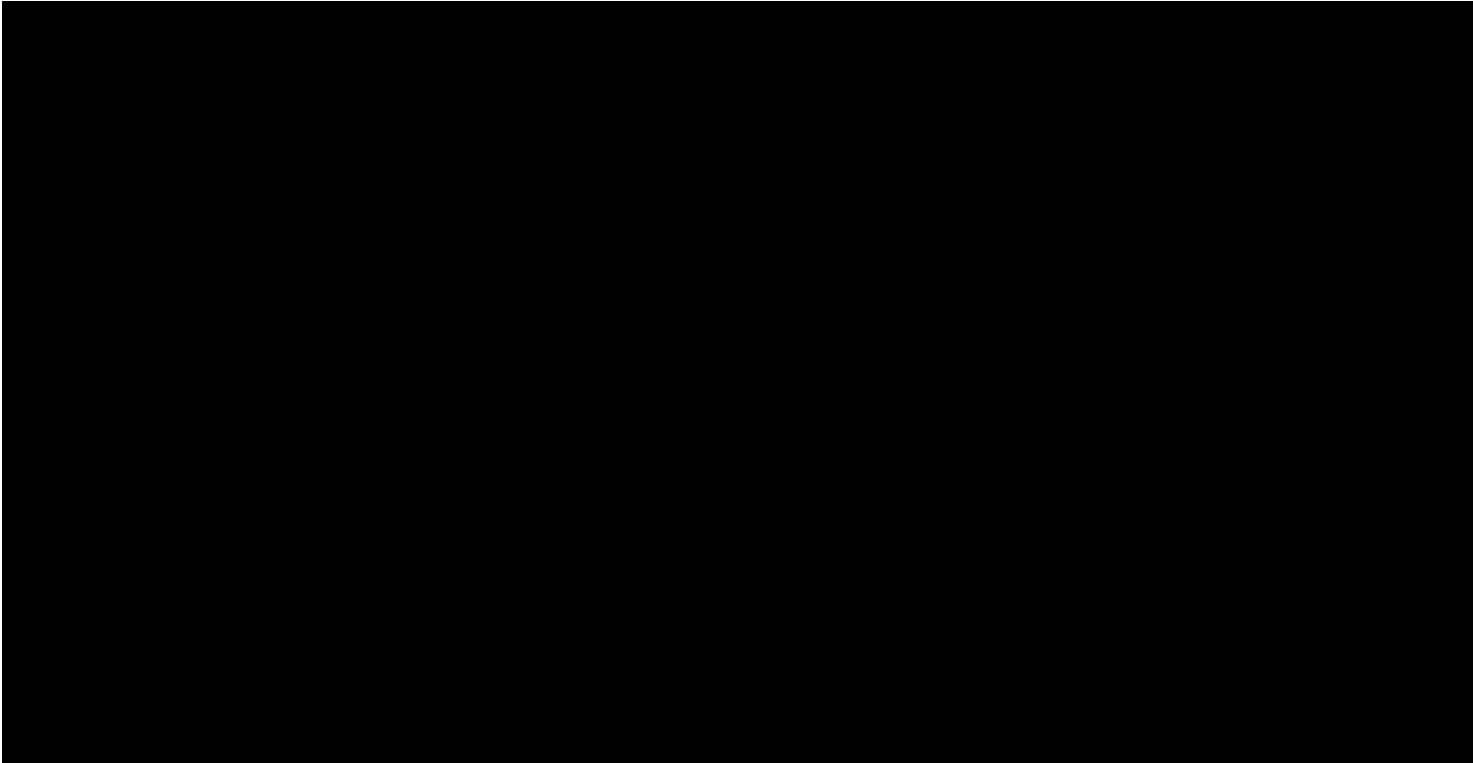
**Table 14.** ASOs from Mr. Watanabe’s Undisclosed Experiment and Structural Features of the ’590 and ’827 Patent Claims

89. Contrary to Dr. Hastings' allegations, researchers from Dr. Wilton's laboratory also continued working on ASOs covering the previously identified hot spot. *See, e.g.*, Hastings Rep. ¶121. While the ASOs additionally tested in Dr. Wilton's laboratory do not fall within the scope of the claims of the Wilton Patents as they were tested as 2'OMePS or MOE ASOs, they nevertheless meet many of the structural requirements of the claims and are reported to induce exon skipping.

90. One example is an ASO targeting the (+23+47) region of human exon 53 with a different backbone chemistry: "MOE" (2'-O-methoxy-ethyl RNA). *See* Westcott Lab Notebook (SRPT-VYDS-0158291-435), pp. 22-24; Chan 2006, Fig. 2;. As reproduced below, the experiment conducted at the end of June 2005 showed a skipped transcript caused by the MOE ASO targeting the (+23+47) region of human exon 53.

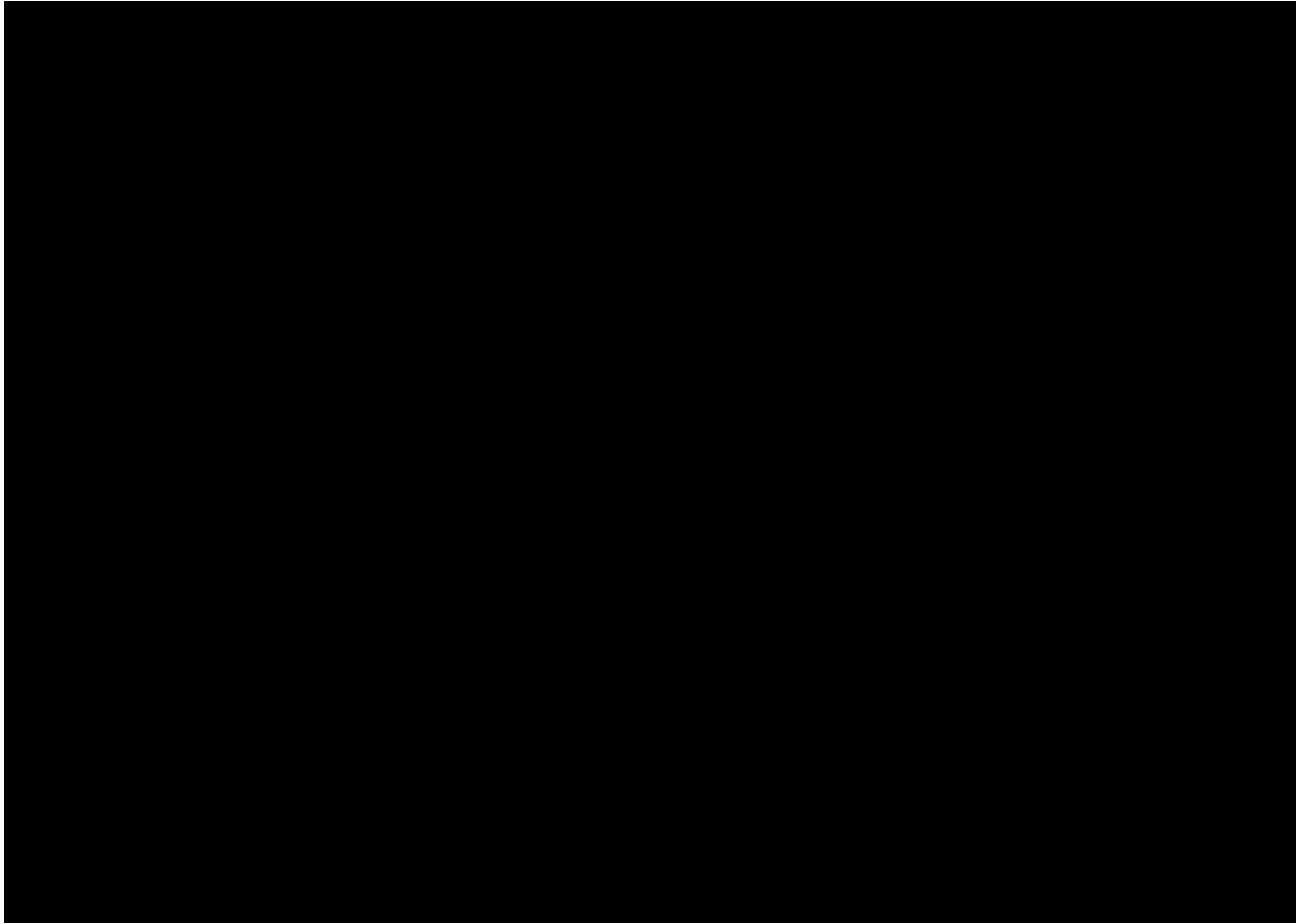


**OUTSIDE COUNSEL EYES ONLY – SUBJECT TO PROTECTIVE ORDER**



91. Both Wilton PCT '350 and additional laboratory notebooks from the Wilton laboratory also record several ASOs directed to the hot spot. *See* Dowdy Op. Rep. § V.C.4.c. Below I have provided notable excerpts from the laboratory notebooks, followed by tables summarizing the work from the Wilton laboratory further confirming that the claimed structural features correlate with inducing exon 53 skipping.

- JSR1979 (ASO targeting the (+27+56) region of human exon 53)<sup>10</sup>



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<sup>10</sup> Ms. Forrest indicates that JSR1979 corresponds to “H53A(+27+57).” Forrest Lab Notebook 4 (SRPT-VYDS-0161827-956), p. 2. The synthesis lab notebook indicates that JSR1979 corresponds to H53A(+27+56), i.e., targeting the (+27+56) region of human exon 53. *See* Jiminy Synthesis Lab Notebook 15 (SRPT-VYDS-0160521-639), p. 34.

- JSR2139, JSR2140, JSR2041 (ASOs targeting the (+34+63), (+33+62), and (+31+61) regions of human exon 53, respectively)





ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region” within nucleotides +23 to +69 of human exon 53	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Wilton '350 (2'OMePS) H53A(+27+56)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+27+56) target region)	Yes (21 consecutive bases)	Yes
Wilton '350 (2'OMePS) H53A(+33+63)	Yes (100% complementary)	Yes (31 mer)	Yes (the (+33+63) target region)	Yes (15 consecutive bases)	Yes
H53A(+23+47) (MOE)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
JSR1979 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+27+56) target region)	Yes (21 consecutive bases)	Yes
JSR2139 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+34+63) target region)	Yes (14 consecutive bases)	Yes
JSR2140 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+33+62) target region)	Yes (15 consecutive bases)	Yes
JSR2141 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+31+61) target region)	Yes (17 consecutive bases)	Yes

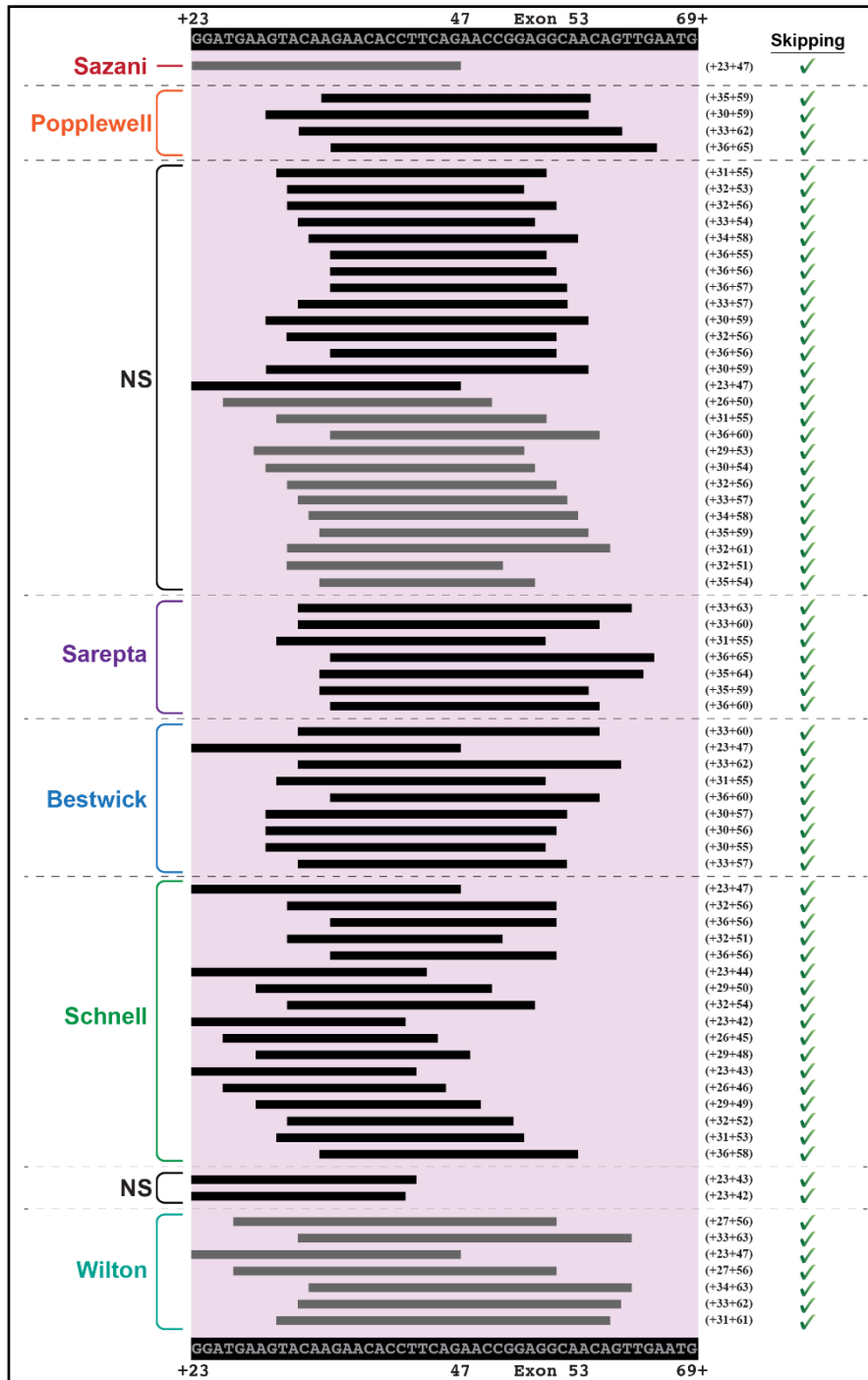
**Table 15.** ASOs from Dr. Wilton’s Laboratory and Structural Features of the ’851 Patent Claims



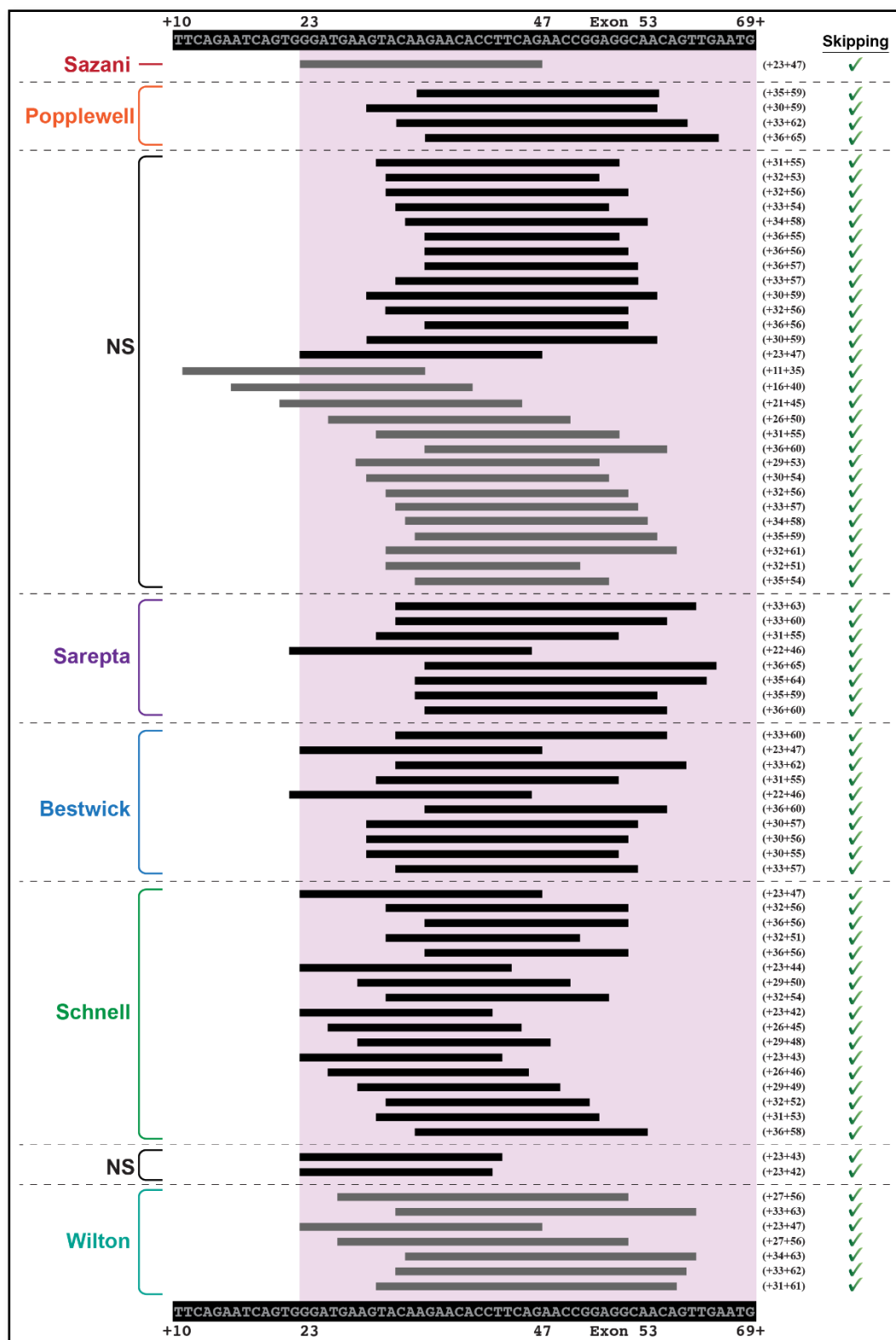
ASO Name (Chemistry)	“antisense oligonucleotide”	“20 to 31 bases”	“a target region”	“at least 12 consecutive bases of [SEQ ID NO: 195]”	Skipping
Wilton '350 (2'OMePS) H53A(+27+56)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+27+56) target region)	Yes (21 consecutive bases)	Yes
Wilton '350 (2'OMePS) H53A(+33+63)	Yes (100% complementary)	Yes (31 mer)	Yes (the (+33+63) target region)	Yes (15 consecutive bases)	Yes
H53A(+23+47) (MOE)	Yes (100% complementary)	Yes (25 mer)	Yes (the (+23+47) target region)	Yes (25 consecutive bases)	Yes
JSR1979 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+27+56) target region)	Yes (21 consecutive bases)	Yes
JSR2139 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+34+63) target region)	Yes (14 consecutive bases)	Yes
JSR2140 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+33+62) target region)	Yes (15 consecutive bases)	Yes
JSR2141 (2'OMePS)	Yes (100% complementary)	Yes (30 mer)	Yes (the (+31+61) target region)	Yes (17 consecutive bases)	Yes

**Table 16.** ASOs from Dr. Wilton’s Laboratory and Structural Features of the ’590 and ’827 Patent Claims

92. The following schematic drawing summarizes the ASOs that I have reviewed in this section. Numerous researchers independently and repeatedly *confirmed* that the claimed structural features of the Wilton Patents confer the claimed function of exon 53 skipping.



**Figure 13.** ASOs from Researchers Sharing Structural Features of the Claims of the '851 Patent Claims and Reported to Induce Exon 53 Skipping (black = PMO, grey = non-PMO)



**Figure 14.** ASOs from Researchers Sharing Structural Features of the Claims of the '590 and '827 Patent Claims and Reported to Induce Exon 53 Skipping (black = PMO, grey = non-PMO)

93. Dr. Hastings ignored *all* of these real-world examples. According to Dr. Hastings, she can do so because “[i]t is my understanding that such post-filing date data, however, cannot be used to affirmatively satisfy the written description requirement as written description requires a specification to reasonably convey to those skilled in the art that inventor had possession of the claimed subject matter as of the alleged priority date.” Hastings Rep. ¶¶88-89; *see also id.*, ¶73 n.10. To be clear, my position is that the specification of the Wilton Patents provides written description support for the claims of the Wilton Patents. I am citing this real-world post-filing evidence to *confirm* that the specification provides adequate written description support and to respond to Dr. Hastings use of post-filing evidence in the form of the CERI Reports.

94. The post-filing date data that I summarized here is not a substitute or supplement of the information disclosed in the specification. Rather, it *validates* what Dr. Wilton and co-inventors disclosed in June 2005 and *confirms* that independent researchers recognized the Wilton Patents’ disclosure that the structural features of the claims of the Wilton Patents result in ASOs that induce exon 53 skipping. *See e.g.*, MDA Press Release dated August 12, 2020 (“Laboratory development of exon-skipping therapies began in the 1990s, including notably with MDA-funded work by Steve Wilton, PhD, and colleagues. Their work led to the invention of what would later become Exondys 51, Vyondys 53, and Viletpso.”).

**d. Dr. Hastings’ Structure-Function Analysis Is Misguided**

95. Notwithstanding the express disclosures and empirical evidence in the specification, Dr. Hastings contends that the Wilton Patents “do not disclose a structural feature common to the members of the claimed genus.” Hastings Rep. ¶61. But Dr. Hastings primarily attempts to discredit the evidence in the specification, in particular, the skipping reported for

H53A(+23+47), and then resorts to her incorrect and overly broad understanding of the claims of the Wilton Patents. *See supra* § V.A.1.a. Dr. Hastings is wrong on both counts.

96. Dr. Hastings' attacks on H53A(+23+47) are unpersuasive. For example, Dr. Hastings appears to contend that H53A(+23+47) (SEQ ID NO: 195) can be ignored because it was made as a 2'OMePS ASO, not a PMO. *See* Hastings Rep. ¶62. But the specification states that all of the disclosed ASOs, including H53A(+23+47), can be made as "morpholino" antisense oligonucleotides. '851 Patent, Table 1A. Further, as discussed above, a POSA would have understood that the exon skipping ability exhibited by a 2'OMePS ASO, including H53A(+23+47), would translate to a corresponding PMO targeting the same region. *See supra* § V.A.2.a. In other words, a POSA would have understood that ASOs with the claimed structural features would induce exon 53 skipping based on the empirical evidence obtained from H53A(+23+47), as well as the additional evidence in the specification discussed above. *See id.* Consistent with this, NS's own experiments later confirmed that a PMO targeting positions 23 to 47 induced exon 53 skipping. '217 Patent, Fig. 18 (reporting skipping induced by PMO No. 16, which corresponds to H53A(+23+47)).

97. Dr. Hastings also notes that H53A(+23+47) induced "very faint" skipping. *See* Hastings Rep. ¶62. But the claims of the Wilton Patents do not require any specific level of exon 53 skipping. A POSA would not have ignored H53A(+23+47) merely because it induced "very faint" skipping, especially when H53A(+23+47) overlaps with other active ASOs that collectively define the exon 53 hot spot. *See supra* §§ V.A.2.a-b.

98. Dr. Hastings then contends that "a POSA cannot envision what structural features are common to all members of the [Wilton] Patent claims based on this *single disclosure* of SEQ ID NO: 195." Hastings Rep. ¶63. But a POSA would have considered the specification as a whole,

**3. The Wilton Patents Disclose a Representative Number of Species**

**a. H53A(+23+47) Is Representative of the Claimed Genera of the Wilton Patents**

108. The claimed genera of the Wilton Patents cover a finite group of PMOs having specific structural features that induce exon 53 skipping. *See supra* §§ V.A.1.b, V.A.2.a. A POSA would have concluded that the specification of the Wilton Patents discloses a representative species of the claimed genera, namely H53A(+23+47).

109. H53A(+23+47) is structurally representative. It shares many structural features with other members of the claimed genera. It is an (1) “antisense” oligonucleotide; (2) it has “20 to 31 bases”; (3) it comprises “a base sequence that is 100% complementary to consecutive bases of a target region of exon 53 of the human dystrophin pre-mRNA”; (4) “the base sequence comprises at least 12 consecutive bases of CUG AAG GUG UUC UUG UAC UUC AUC C (SEQ ID NO: 195)”; and (5) in the case of the ’851 Patent, its “target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69).” Although H53A(+23+47) is a 2’OMePS ASO with uracil bases, the specification states that H53A(+23+47) can be made as a “morpholino” antisense oligonucleotide with thymine bases in place of uracil bases. *See* ’851 Patent, Table 1A.

110. H53A(+23+47) is also functionally representative because it “induces exon 53 skipping.” In view of the general knowledge in the art, a POSA would have understood that the exon skipping ability exhibited by a 2’OMePS “H53A(+23+47)” ASO would generally translate to a corresponding “H53A(+23+47)” PMO. *See supra* § V.A.2.a; *see* Gebiski 2003, 1805; Fletcher 2006, 208; Adams 2007, 6.

111. Subsequent studies from researchers around the world, ignored by Dr. Hastings, confirm that H53A(+23+47) is representative of the claimed genera of the Wilton Patents. *See supra* § V.A.2.c. These studies repeatedly confirmed that ASOs sharing the structural features of

H53A(+23+47), including H53A(+23+47) made as a PMO, induced exon 53 skipping. *Id.* Two notable examples are NS's viltolarsen and Sarepta's golodirsen. *Id.* As illustrated below, H53A(+23+47) is representative of these compounds.

	H53A(+23+47)	Viltolarsen	Golodirsen
"antisense oligonucleotides"	✓	✓	✓
"20 to 31 bases"	✓	✓	✓
"a base sequence that is 100% complementary to consecutive bases"	✓	✓	✓
"a target region of exon 53 of the human dystrophin pre-mRNA"	✓	✓	✓
"at least 12 consecutive bases of [SEQ ID NO: 195]"	✓	✓	✓
"morpholino" w/ "thymine"	X (Table 1A states that this ASO can be made as "morpholino" with "T")	✓	✓
"the target region is within annealing site H53A(+23+47) and annealing site H53A(+39+69)"	✓	✓	✓
"induces exon 53 skipping"	✓	✓	✓

**Table 17.** Representativeness of H53A(+23+47) as Compared to Viltolarsen and Golodirsen

112. In sum, a POSA reading the specification of the Wilton Patents would have recognized that H53A(+23+47) is representative of the claimed genera of the Wilton Patents, which encompass PMOs sharing structural characteristics that correlate with exon 53 skipping.

**b. Dr. Hastings' Analysis Ignores the Representative Aspects of H53A(+23+47)**

113. Dr. Hastings asserts that H53A(+23+47) cannot be representative of the claimed genera of the Wilton Patents. *See* Hastings Rep. § IX.A. Dr. Hastings lists several reasons: "the seemingly infinite number of antisense oligonucleotides encompassed by the [Wilton] Patent claims," "the sole disclosure of SEQ ID NO: 195," "the unpredictability in the field of exon



skipping, and the named inventors' own admission in the specification of the [Wilton] Patents, as well as in subsequent publications and at deposition.” *Id.*, ¶¶60. I disagree for the reasons set forth below.

**i. “Seemingly Infinite Number”**

114. Dr. Hastings asserts that “the sole disclosure of SEQ ID NO: 195 is insufficient to support the broad genus of up to  $10^{14}$  possible antisense oligonucleotides encompassed by the claims of the [Wilton] Patents.” Hastings Rep. ¶¶49-50. Both her premise and conclusion are wrong. When appropriately analyzed, the scope of the claims of the Wilton Patents is much narrower, and a POSA would have been able to visualize the members of the claimed genera of PMOs based on the common structural features identified in the specification and required by the claims. *See supra* §§ V.A.1.b, V.A.2.a. In that context, the disclosure of H53A(+23+47) is highly relevant, as it has the structural and functional properties of the claimed genera, the specification teaches that it can be made as a PMO, and it targets the exon 53 hotspot. *See supra* § V.A.3.a.

**ii. “Sole Disclosure of SEQ ID NO: 195”**

115. Dr. Hastings criticizes H53A(+23+47) for being a 2'OMePS ASO with uracil bases, not a PMO with thymine bases. *See* Hastings Rep. ¶50. But the specification states that H53A(+23+47) can be made as a “morpholino” antisense oligonucleotide with thymine bases in place of uracil bases. *See* '851 Patent, Table 1A. Further, a POSA would have understood that the skipping activity of H53A(+23+47) would generally translate to a corresponding PMO, as contemporaneous studies demonstrated for other exon skipping ASOs. *See supra* § V.A.2.a; GebSKI 2003, 1805; Fletcher 2006, 208; Adams 2007, 6. Subsequent research also confirmed that a PMO targeting the same region as H53A(+23+47) induces exon 53 skipping. *See supra* § V.A.2.c.

Dowdy Op. Rep. §§ IV.B (Claim Construction), VII.B. (The Asserted Claims of the Wilton Patents). The Wilton Patents state that H53A(+23+47) “induces exon 53 skipping” and thus meets the functional requirement of the claims. *See* ’851 Patent, Table 39.

118. Relatedly, Dr. Hastings states that “[t]here is no disclosure in the [Wilton] Patents . . . that shows the inventors were in possession of a genus of antisense oligonucleotides having a therapeutic effect.” Hastings Rep. ¶59. But the claims of the ’851 and ’590 Patents are directed to antisense oligonucleotides, not treatment. *See* Dowdy Op. Rep. §§ VII.B.1-2. While the claims of the ’827 Patent are directed to a method for treating a patient with DMD, the claims also do not require any particular degree of therapeutic efficacy. *See id.*, §§ VII.B.3; IV.B (Claim Construction).

119. Notably, as Dr. Wilton explained, at least two PMOs that fall within the claim scope of the Wilton Patents are now FDA-approved for treating DMD in patients with a mutation that is amenable to exon 53 skipping. *See* Dowdy Op. Rep. §§ VI, IX; Wilton Tr. 21:16-20 (“Q. Did you invent golodirsen? **A. Golodirsen was derived from the coordinates we had identified in our original 2005 patent.**”), 31:12-15 (“Q. Did you identify the precise coordinates of viltolarsen? **A. We defined the boundaries where viltolarsen would be found.**”). Dr. Hastings does not identify any ASO covered by the claims of the Wilton Patents that was tested in humans that did not show a “therapeutic effect,” and I am aware of none.

### iii. “Unpredictability in the Field of Exon Skipping”

120. As in her structure-function analysis, Dr. Hastings relies on Dr. Wood’s generalized assertion that “the deletion of as few as two nucleotides from an antisense oligonucleotide that induces exon skipping or changes to a significant number of nucleotides in an antisense oligonucleotide that induces exon skipping can reduce or eliminate such activity altogether.” Hastings Rep. ¶54 (citing Wood Rep. ¶81, Wood Interference Decl. ¶74); *id.*, ¶51 (citing Wood

Rep. ¶¶75-86, Wood Interference Decl. ¶¶68-77). Dr. Hastings then contends that “[i]n view of the unpredictability associated in changing the number of nucleotides in an antisense oligonucleotide, and in particular with shortening antisense oligonucleotides, a POSA would not conclude, based on the sole disclosure of SEQ ID NO: 195, that the named inventors were in possession of a genus of antisense oligonucleotides shorter or longer than SEQ ID NO: 195, that comprise at least 12 consecutive bases of SEQ ID NO: 195, that still exhibit exon 53 skipping.” Hastings Rep. ¶56. I disagree.

121. Unpredictability Generally: As discussed above, Dr. Wood’s “unpredictability” opinions are not focused on exon 53. See *supra* § V.A.2.d. Dr. Wood largely discusses general unpredictability in the field *not* tied to exon 53, including experiments evaluating ASOs targeting other exons as well as non-human dystrophin. See Wood Rep. ¶¶75-79 (quoting statements regarding general unpredictability), ¶80 (ASOs targeting mouse exon 46), ¶81 (ASOs targeting human exon 50 and two flanking intron sequences), ¶¶84-85 (ASOs targeting mouse exon 23), ¶86 (ASOs targeting human exon 51). While Dr. Hastings also cites Dr. Wood’s declaration submitted to the USPTO during an interference proceeding for additional support, that declaration makes the same arguments citing the same evidence and is therefore similarly flawed. See Wood Interference Decl. ¶¶68-72 (quoting statements regarding general unpredictability), ¶73 (ASOs targeting mouse exon 46 discussed in ¶80 of Wood Rep.), ¶74 (ASOs targeting human exon 53 and two flanking intron sequences discussed in ¶81 of Wood Rep.), ¶¶75-76 (same ASOs targeting murine exon 23 discussed in ¶¶84-85 of Wood Rep.), ¶77 (ASOs targeting human exon 51 discussed in ¶86 of Wood Rep.).

122. Critically, neither Dr. Wood nor Dr. Hastings address the pertinent question of whether a POSA would have believed that ASOs within the scope of the claims would

unpredictably induce exon 53 skipping *after* reading the specification. *See supra* §§ III.B, V.A.2.d. Here, as discussed above, the specification discloses a group of overlapping ASOs that induced exon 53 skipping. '851 Patent, Table 39; *see supra* §§ V.A.2.a-b. Once that hot spot was identified, a POSA reading the specification would have reasonably expected that additional ASOs directed to regions near to or within the hot spot, and sharing the structural features claimed by the Wilton Patents, would similarly induce exon 53 skipping. *See id.*; Wilton Tr. 58:3-13 (Dr. Wilton explaining that “[b]ased on our experience, all oligonucleotides we designed to that region will induce some exon skipping – exon 53 skipping”). Subsequent studies, ignored by Dr. Hastings, confirmed that other members of the claimed genera similarly induce exon 53 skipping. *See supra* § V.A.2.c. Dr. Hastings does not identify any real-world examples of ASOs that shared structural features of H53A(+23+47) and did not induce exon 53 skipping.<sup>14</sup> *See* Hastings Rep. ¶¶50-60.

123. Unpredictability Relating to Length: Dr. Hastings contends that H53A(+23+47), which is 25 bases in length, cannot be representative of PMOs spanning 20 to 31 bases in length because “the named inventors had [not] identified an optimal length for antisense oligonucleotides for exon 53 skipping.” Hastings Rep. ¶57; *id.*, ¶55 (discussing Harding 2007 and quoting Dr. Wilton’s testimony discussing an “optimum” length); *see also* Wood Rep. ¶82 (asserting that “AONs have an optimal length”).

124. Dr. Hastings again imposes a limitation absent in the claims of the Wilton Patents. The claims do not require an “optimal” length or “optimal” degree of exon skipping. Instead, they require that each claimed PMO is “20 to 31 bases” in length and that the PMO “induces exon 53 skipping.” *See* Dowdy Op. Rep. §§ IV.B (Claim Construction), VII.B. (The Asserted Claims of

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<sup>14</sup> Again, Dr. Hastings may point to some of the ASOs tested by the CERI. *See* Hastings Rep. ¶¶90-113. As discussed below, these artificially designed ASOs do not fall within the scope of the claims of the Wilton Patents. *See infra* §§ V.A.4.c.ii-iii.

the Wilton Patents). H53A(+23+47) is 25 bases in length—and therefore is “20 to 31 bases” in length—and is reported to induce exon skipping. ’851 Patent, Table 39. Three other ASOs that overlap with H53A(+23+47), namely H53A(+39+62), H53A(+45+69), and H53A(+39+69), are 24, 25, and 31 bases in length, respectively, are reported to induce exon skipping. *Id.* The specification tested additional exon 53 skipping ASOs spanning 20 to 31 bases in length and identifies “20-31 bases” as a general length of ASOs that can induce exon skipping. *Id.*, Table 39, 23:63-66. While omitted by Dr. Hastings, Dr. Wilton testified that he considered 20 to 31 bases in length as “the optimum length of oligonucleotides within the defined region we’ve identified from plus 23 to plus 69.” Wilton Tr. 98:11-18. A POSA would have concluded that H53A(+23+47), having 25 bases, is representative of claimed PMOs that are 20-31 bases in length and that the inventors were in possession of such PMOs.

125. Dr. Hastings also cites Harding 2007 as evidence of the alleged unpredictability associated with ASO length. *See* Hastings Rep. ¶55; *see also* Wood Rep. ¶82 (discussing Harding 2007). Harding 2007 reports that “[o]verlapping 25mers were only able to induce very low levels of exon 53 skipping, whereas the 31mer induced consistent exon skipping at transfection concentrations as low as 10 nM.” Harding 2007, 164. It is clear from this disclosure that, despite having varying lengths, all of these ASOs induced exon 53 skipping. *Id.*, Figure 4(b). Further, Harding 2007 does not state or suggest that ASOs shorter than 25 bases in length did not induce exon 53 skipping. Dr. Wilton in deposition also expressly rejected that notion. Wilton Tr. 97:8-98:18 (Dr. Wilton explaining that ASOs having 20 to 31 bases in length would be optimal for the identified hot spot).

126. Further, even if changing ASO length by a few bases can impact the level of exon skipping as a general matter, that does not indicate that ASOs having the claimed structural features

would not induce exon 53 skipping. As Dr. Wood explains, and I agree, the length of an ASO works in concert with a number of other factors, “including nucleotide sequence, chemical modifications, and target accessibility” to confer skipping ability to the ASOs. *See* Wood Rep. ¶82. Here, the claims of the Wilton Patents specify each of these factors: “20 to 31 bases” (the length), “a target region of exon 53 of the human dystrophin pre-mRNA,” (target accessibility), “a base sequence that is 100% complementary to consecutive bases” of that target region and comprises “at 12 consecutive bases” of SEQ ID NO: 195 (the nucleotide sequence), and “morpholino” (the chemical modification). And the post-filing evidence that I have reviewed to date (including the artificially designed experiments conducted by the CERI) confirms the teaching of the specification: that ASOs incorporating the claimed features consistently induce exon 53 skipping, undercutting the general unpredictability. *See supra* §§ V.A.2.c, V.A.4.c.ii-iii; *see* Hastings Rep. ¶106 (e.g., PMO-9, PMO-12, PMO-13, PMO-14, PMO-15, PMO-16, all of which fall within the claim scope of the Wilton Patents and induced exon 53 skipping).

127. For the same reason, Dr. Hastings’ assertion that “a POSA would not consider that the named inventors were in possession of a genus of antisense oligonucleotides having exon skipping activity merely because they shared 12 consecutive bases of SEQ ID NO: 195” is meritless. Hastings Rep. ¶51. The claimed genera of the Wilton Patents share more than 12 consecutive bases of SEQ ID NO: 195—they share “antisense” (the level of complementarity), “20 to 31 bases” (length), “a target region of exon 53 of the human dystrophin pre-mRNA” (the target region), “a base sequence that is 100% complementary to consecutive bases” of that target region (the base sequence), and “morpholino” (the chemistry). Again, Dr. Hastings fails to take into account all of the claim limitations of the Wilton Patents, and therefore fails to properly analyze the representativeness of H53A(+23+47) in that context.

128. Dr. Hastings also implies that the recitation of “at least 12 consecutive bases of SEQ ID NO: 195” is not important to exon skipping. Hastings Rep. ¶¶52-53. But, as Dr. Hastings acknowledges, the specification expressly states: “With some targets such as exon 19, antisense oligonucleotides as short as 12 bases were able to induce exon skipping, albeit not as efficiently as longer (20-31 bases) oligonucleotides.” ’851 Patent, 23:63-66. As Dr. Wilton explained, a POSA reviewing the results reported in Table 39 in light of this statement would have recognized that the limitation “at least 12 consecutive bases” of SEQ ID NO: 195 was “central” to the exon 53 hot spot disclosed in the Wilton Patents, especially when incorporated as “the crucial part of the splice switching oligonucleotides,” which have a “sufficient length containing 12 bases from this sequence.” Wilton Tr. 228:10-18, 230:10-21, 231:24-232:16. The criticality of this limitation is also confirmed by many ASOs made and tested by many researchers, which included 12 or more consecutive bases of SEQ ID NO: 195 along with other claimed structural features and induced exon skipping. *See supra* § V.A.2.c.

**iv. “Named Inventors’ Own Admission”**

129. Pointing to statements in the specification, Dr. Hastings asserts that “by their own admission, the named inventors did not consider the rational design methodologies used in the specification’s Examples to be ‘reliable.’” Hastings Rep. ¶58. The statements that Dr. Hastings cite are reproduced below:

Attempts by the inventors to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore most therapeutic antisense molecules compounds has been the result of empirical studies.

These empirical studies involved the use of computer programs to identify motifs potentially involved in the splicing process. Other computer programs were also used to identify regions of the pre-mRNA which may not have had extensive secondary structure and

#### **4. Response to Dr. Hastings' Other Arguments**

132. After offering her opinions on a “representative number of species” and “a structural feature common to the members of the claimed genus,” Dr. Hastings raises certain arguments relating to the exon 53 hot spot identified by Dr. Wilton and his co-inventors, some laboratory notebooks from Dr. Wilton’s laboratory, and the testing performed by CERI. These additional arguments are addressed below.

##### **a. Dr. Hastings' Improperly Dismisses the Significance of the Identification of the Hot Spot on which the Claimed Structural Features Are Based**

133. As illustrated above, the claims of the Wilton Patents structurally define a limited group of PMOs that are directed to a discrete region within exon 53 of the human dystrophin pre-mRNA. *See supra* §§ V.A.1.b, V.A.2.a. The claimed inventions are based on the breakthrough discovery of Dr. Wilton and co-inventors, who, for the first time, recognized a hot spot within exon 53. *See supra* § V.A.2.b; Dowdy Op. Rep. § V.C.4.b. Dr. Hastings dismisses the significance of the work reported in the Wilton Patents, contending that “[p]ost-priority date evidence supports that Dr. Wilton and his co-inventors neither recognized nor appreciated the +23 to +69 region of exon 53 to be a ‘hotspot.’” Hastings Rep. § IX.C. I disagree.

134. First and foremost, Dr. Hastings completely ignores the direct evidence available in this case, reflecting what a POSA would have understood from reading the specification. As noted above, researchers at Sarepta recognized long before this litigation that the Wilton Patents disclosed an “effective target region of +23 to +69 relative to the splice acceptor site.” Sequence Selection Report, SRPT-VYDS-0201529; Dowdy Op. Rep. § VI.A. Indeed, the hot spot identified by Dr. Wilton and his co-inventors directly influenced the selection of candidate PMOs. Sequence Selection Report, SRPT-VYDS-0201531 (“All sequences are internal to Exon 53 and fell within the previously identified effective region (+23+69).”). The candidate PMOs targeting the hot spot



were blindly tested at multiple testing sites, ultimately leading to the identification of golodirsen as Sarepta's clinical candidate. *Id.*, SRPT-VYDS-0201531-33.

135. Ignoring this highly relevant evidence, Dr. Hastings instead isolates phrases from publications and email correspondences, none of which supports Dr. Hastings' allegation. For instance, Dr. Hastings points to a publication from Dr. Wilton (Harding 2007) and an email from Ms. Harding to Dr. Wilton (WILTON0026361-67), alleging that "Dr. Wilton characterized the 'hotspot' as '39–69 bases within the 212 base long exon 53.'" Hastings Rep. ¶67. Dr. Hastings mischaracterizes these documents. The term "hotspot" is mentioned a total of three times in Harding 2007 and the email. As reproduced below, in all three instances, the term is used to describe a group of exons within the human dystrophin gene that are highly mutated and cause DMD (*see* Dowdy Op. Rep. ¶91)—*not* a region of exon 53 amenable to exon skipping:

The most common type of dystrophin mutation is a genomic deletion of one or more exons, occurring predominately in two **hotspots** involving the 5' end and exons 44–55. (Harding 2007, 157.)

Exon 51 has been chosen for the "first time in human" studies, as removing this exon could restore the reading frame in a substantial number of DMD patients who have deletions in the major mutation **hotspot** of the dystrophin gene. (Harding 2007, 159.)

The dystrophin gene is one of the largest known and has 2.4 million base pairs and 79 exons (Roberts). The most common type of mutation in dystrophin is a genomic deletion of one or more exons. These are observed in two **hot spots** involving exons 3 to 7 and 44 to 55. (WILTON0026361-67.)

136. In other words, the term "hot spot" was used in these documents for something unrelated—mutations that cause disease. In contrast, the invention of the Wilton Patents focuses on a "hot spot" amenable to exon skipping by ASOs to *treat* DMD.

137. To the extent that Dr. Hastings' allegation is based on the statement in Harding 2007 that "amenable sites to redirect dystrophin splicing were identified at . . . 39-69 bases within

the 212 base long exon 53,” I again fail to see how this statement contradicts the earlier identification of the hot spot. Harding 2007, 164. Dr. Wilton and co-inventors identified the hot spot of nucleotides +23+ to +69 based on empirical testing. That a later publication characterizes a region falling within the hot spot as “amenable” to skipping is consistent with the prior identification of the hot spot.

138. Dr. Hastings then asserts that these documents “all indicate that [Dr. Wilton’s] exon 53 optimization efforts were focused on the +39 to +69 region, not +23 to +69.” I disagree. First, this is factually incorrect. The specification of the Wilton Patents itself discloses an ASO targeting the (+23+47) region of human exon 53. *See* ’851 Patent, Table 1A. The work from Dr. Wilton’s laboratory also shows that Dr. Wilton and researchers continued evaluating ASOs directed to the entire hot spot. *See supra* § V.A.2.c; *see, e.g.*, Forrest Lab Notebook 4, pp. 2-5 (evaluating two ASOs respectively targeting the (+27+56) and (+27+60) regions of exon 53).

139. Second, Dr. Hastings improperly conflates later “optimization efforts” with the invention of the Wilton Patents. That Dr. Wilton sought to optimize an ASO targeting the (+39+69) region of human exon 53 *after* identifying the hot spot does not undermine the earlier disclosure of the +23 to +69 hotspot. *See supra* § V.A.2.b.

140. For example, Dr. Hastings contends that “Ms. Adams considered H53A(+39+69) to be their ‘optimized exon 53 AO’ as of November 2005” and that “Ms. Adams then conducted a ‘walk’ around the +39+69 coordinates in December 2007.” Hastings Rep. ¶68. As Dr. Wilton explained, however, once the hot spot was identified, it was natural to look for “oligos that induce equivalent, better, or worse exon skipping.” Wilton Tr. 55:21-56:20. That Ms. Adams conducted experiments to optimize exon skipping of a particular antisense oligonucleotide that falls within the hot spot after June 2005 does not negate the hot spot disclosed in the Wilton Patents. Nor, as

Dr. Hastings contends, does it show that the inventors “did not view the ‘hot-spot’ for exon 53 to include sequence upstream (5’) of +39 at this time.” *See* Hastings Rep. ¶68.

141. Dr. Hastings also discusses events around 2008 to 2010, contending that “[f]rom the summary of Ms. Adams’ exon 53 optimization efforts, it is apparent that she did not design or test any antisense oligonucleotides that went closer to +23 to +47.” Hastings Rep. ¶69. Again, this confuses downstream “optimization” with the identification of the hot spot and the disclosure of the Wilton Patents. It is also incorrect. As noted above, in July 2010, Ms. Forrest, one of the researchers in Dr. Wilton’s laboratory, tested two ASOs respectively targeting the (+27+56) and (+27+60) regions of exon 53. Both induced skipping. *See* Forrest Lab Notebook 4, pp. 2-5.

142. If anything, the documents cited by Dr. Hastings reveal contemporaneous appreciation of the hotspot. As discussed in my Opening Expert Report, Popplewell 2010 reported several effective 25-mer and 30-mer PMOs, spanning from nucleotides +30 to +74 of human exon 53. *See* Dowdy Op. Rep. § X.B.1.a. After receiving a draft of Popplewell 2010, Dr. Wilton commented that Drs. Popplewell and Dickson “decided to microwalk an area because it was in a hybridization zone” and that an ASO from Dr. Wilton’s laboratory “was in the middle of that zone.” WILTON0017692-95, -94. This comment reflects contemporaneous recognition that the PMOs evaluated in Popplewell 2010 fell within the hot spot reported in the Wilton Patents.

143. Dr. Hastings also points to an email from Dr. Wilton dated July 28, 2010, allegedly showing “a lack of recognition or appreciation by the inventors that the ‘hot-spot’ extended to +23 at any time, much less before the priority date of the [Wilton] Patents.” Hastings Rep. ¶70 (citing WILTON0018072). The email states: “[t]he hot spot being targeted is 27 bases in from the start of the exon to 72 bases from the [start] of the exon” and “[w]e have numerous overlapping oligos of different lengths targeting this region.” WILTON0018072-75, -74. Contrary to Dr. Hastings’

interpretation, Dr. Wilton's comment supports my assessment. Even after 5 years from the initial disclosures of the Wilton Patents, Dr. Wilton continued to investigate the hot spot identified as of June 2005 and to optimize ASOs falling within that hot spot.

144. Dr. Hastings then turns to select work and statements from Sarepta, alleging that "they too did not recognize that the inventors of the [Wilton] Patents had identified a 'hotspot' skipping region spanning positions +23+69 of exon 53." Hastings Rep. ¶¶71-72. But, as discussed above, Dr. Hastings' allegation disregards the statement from Sarepta's scientists, made in 2013-14, that the Wilton Patents "identified [an] effective target region of +23 to +69 relative to the splice acceptor site." Sequence Selection Report, SRPT-VYDS-0201529.

145. Further, Dr. Hastings' allegation is difficult to follow. For example, Dr. Hastings suggests that Drs. Sazani and Kole must have designed PPMOs that "targeted the entirety of exon 53" because they did not believe in the hot spot identified by Dr. Wilton and co-inventors. Hastings Rep. ¶71. This is pure speculation. *See* Sazani PCT '586, pp. 75-76. Regardless, the work of Drs. Sazani and Kole *confirms* the hot spot identified by Dr. Wilton and co-inventors (and supports the asserted claims of the Wilton Patents) by independently demonstrating that an ASO targeting the (+23+47) region of exon 53 induced exon skipping. *See supra* § V.A.2.c; Dowdy Op. Rep. ¶¶103-105.

146. Dr. Hastings also states that Sarepta in the IPR proceedings argued that the specification of the Wilton Patents "demonstrated that AOs targeting the (+30+74) region of exon 53 induce exon skipping." Hastings Rep. ¶72. According to Dr. Hastings, she is "unable to reconcile how Sarepta could argue in the related IPRs that the [Wilton] Patents disclosed a 'hotspot' region spanning positions +30+74, and yet, in the present litigation, argue that the 'hotspot' disclosed by the same patent specification has now shifted upstream to positions +23+69

(b) Conclusion Drawn from the Results

210. Notwithstanding these design flaws, Dr. Hastings asserts that “[t]hese data show that there is no structure-function relationship between exon 53-skipping and the structural features claimed in the [Wilton] Patents.” Hastings Rep. ¶107. I disagree. As explained below, the data further confirm that the Wilton Patents provide adequate written description support for the claimed inventions.

211. First, *all* of the experimental oligonucleotides that Dr. Hastings “designed” that fall within the scope of the claims of the Wilton Patents induced exon 53 skipping. Second, the experimental oligonucleotides that Dr. Hastings designed that do not fall within the scope of the claims of the Wilton Patents showed “No” skipping. In other words, contrary to Dr. Hastings’ assertions, there is a near perfect “structure-function relationship between exon 53-skipping and the structural features claimed in the [Wilton] Patents.” *See* Hastings Rep. ¶107.

0644 CERI Report				
Tested Oligonucleotide	Length	Design Rationale	Skipping	In Claim Scope

[REDACTED]

[REDACTED]

212. Notably, Dr. Hastings also used PMOs having the same target regions that Dr. Wilton and co-inventor identified in Table 39 as either “positive” or “negative” controls. The skipping activities of these PMOs perfectly matched with the 2’OMePS ASOs reported in Table 39. This again confirms that a POSA reading the specification would have understood that the inventors were in possession of PMOs targeting the identified hot spot, even though the identification was based on 2’OMePS ASOs.

0644 CERI Report				Wilton Patents	
Tested Oligonucleotide	Length	Description	Skipping	ASO	Skipping
[REDACTED]					

**Table 21.** Consistency Between 2’OMePS ASOs in the Wilton Patents and PMOs Tested in the 0644 CERI Report (adapted from Hastings Rep. ¶106)

213. I understand that written description must be analyzed from the perspective of a POSA as of the priority date. *See supra* § III.B. The 0644 CERI Report confirms that if a POSA were to follow the teachings of the Wilton Patents, in view of the general knowledge in the art, a POSA would have been able to successfully make exon 53 ASOs that fall within the scope of the claims of the Wilton Patents. Purposefully departing from the guidance in the Wilton Patents, and

thereby making oligonucleotides outside of the scope of the claims, results in compounds that do not induce exon skipping. Thus, the 0644 CERI Report confirms that the Wilton Patents provide written description support for the claimed inventions.

**iii. 0661 CERI Report**

**(a) Dr. Hastings' Design Rationale Behind Tested Oligonucleotides**

214. Like the 0644 CERI Report above, Dr. Hastings states with respect to the 0661 CERI Report: “I designed these experiments to provide more data regarding whether and how exon skipping activity varies across the variety of different PMOs meeting the structural limitations of the claimed genus.” Hastings Rep. ¶101. The following table summarizes morpholino oligomers tested by CERI in the 0661 CERI Report and their corresponding design categories (1) through (4) (as listed above):

0661 CERI Report

**Table 22.** Morpholino Oligonucleotides Tested in the 0661 CERI Report  
(Hastings Rep. ¶¶106, 110)

215. For the same reasons discussed above, PMO-R5 and PMO-R6, which contain “random” bases instead of complementary bases, are not within the scope of the claims of the Wilton Patents. *See supra* § V.A.1.a. Indeed, as discussed above, “PMO-R6” is 100%

0661 CERI Report				
Tested Oligonucleotide	Length	Design Rationale	Skipping	In Claim Scope

**Table 23.** Summary of 0661 CERI Report  
(adapted from Hastings Rep. ¶110)

219. Notwithstanding these critical design flaws, these oligonucleotides offer some meaningful insights. *First*, they again underscore the importance of the claimed structural features of the Wilton Patents and their correlation with the claimed function of inducing exon 53 skipping. *Second*, the oligonucleotides with “random” bases empirically demonstrate the importance of achieving high complementarity, as taught in the specification. *Third*, among the alleged “weasel” compounds that Dr. Hastings designed, [REDACTED]

[REDACTED]. Unlike the other compounds that did not induce exon skipping, these skipping compounds target two segments within human exon 53 that are relatively close to one another and either fall within or are near to the hot spot. These compounds further highlight the importance of the hot spot identified by Dr. Wilton and his co-inventors.

220. In sum, the Wilton Patents provide adequate written description support for the claimed inventions of the Wilton Patents, both by disclosing structural features common to the members of the genus such that a POSA can visualize or recognize members of the genus, and by



excipient such as water or saline solutions. *Id.*, 28:45-29:14. The specification also teaches that pharmaceutical compositions containing ASOs can be administered by any means known in the art, including an intravenous route of administration. *Id.*, 29:15-22.

249. In sum, the claims and the specification of the Wilton Patents provide extensive guidance for designing, synthesizing, and testing claimed ASOs and using them for treating DMD in human patients amenable for exon 53 skipping. Reading the claims and the specification of the Wilton Patents in light of the general knowledge in the art, a POSA would be able to successfully make and test claimed ASOs for the purpose of treating DMD in patients amenable for exon 53 skipping. Indeed, many researchers succeeded in doing so since the Wilton Patents were first published, including researchers from NS and Sarepta. *See supra* § V.A.2.c; Dowdy Op. Rep. §VI.

**ii. Dr. Hastings Ignores Both the Claims and the Specification of the Wilton Patents**

250. Dr. Hastings asserts that the specification lacks sufficient guidance or direction for two alleged reasons. *First*, Dr. Hastings asserts that “the inventors expressly acknowledged that [certain] techniques failed to design antisense oligonucleotides that reliably induced exon 53 skipping.” Hastings Rep. ¶118. *Second*, Dr. Hastings also asserts that “the [Wilton] Patents do not provide guidance for using SEQ ID NO: 195 as a starting point for the development of additional antisense oligonucleotides.” Hastings Rep. ¶¶118, 120; *id.*, ¶121 (“There simply is no teaching in the [Wilton] Patents for selecting SEQ ID NO: 195 to develop additional antisense oligonucleotides for any purpose, let alone for therapeutic use.”). I disagree.

251. As an initial matter, Dr. Hastings’ analysis is incomplete. I understand that the question at issue is whether a POSA *reading* the Wilton Patents would be able to make and use the *claimed inventions* of the Wilton Patents without undue experimentation. *See supra* § III.C.

unassisted and non-conjugated PMO has been demonstrated”), Table 1 (summarizing publications evaluating PMOs in animal models), Table 3 (summarizing PMOs in human clinical trials for treating cancer and West Nile Virus). A POSA would have expected that H53A(+23+47) and other 2’OMePS ASOs targeting the hot spot of exon 53 disclosed in the specification would induce exon 53 skipping at a similar, if not greater, degree, if they were used as PMOs *in vivo*.

264. In sum, the specification discloses several working examples that a POSA would be able to follow in making and using additional ASOs within the scope of the claims. Dr. Hastings’ dismissal of these working examples solely because they were evaluated as 2’OMePS ASOs with uracil bases is improper.

**e. The Predictability of the Art**

265. Dr. Hastings’ unpredictability analysis suffers from the same deficiency discussed above. *See supra* §§ V.A.2.d, V.A.3.b.iii. By focusing on general unpredictability in the art, Dr. Hastings (and Dr. Wood) fails to consider the disclosures of the Wilton Patents in context. In my opinion, once the work of Dr. Wilton and co-inventors reported in the Wilton Patents became available to a POSA, a POSA would have reasonably expected that ASOs having the claimed structural features would induce exon 53 skipping.

266. Similar to her written description opinions, Dr. Hastings primarily relies on Dr. Wood’s opinions and contends that “the ability of antisense oligonucleotides to induce dystrophin pre-mRNA exon skipping is unpredictable.” Hastings Rep. ¶¶116-117 (citing Wood Rep. ¶¶75-89, 193<sup>35</sup> and Wood Interference Decl. ¶¶68-81). Both Dr. Hastings and Dr. Wood, however, fail to address what a POSA would have understood *after* reviewing the Wilton Patents. Indeed, although the art of exon skipping antisense oligonucleotide was generally unpredictable, the

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<sup>35</sup> As noted above, I assume Dr. Hastings meant to cite paragraph 139 as there is no paragraph 193 in Dr. Wood’s report.

Wilton Patents identified, for the first time, a discrete region within exon 53 of the human dystrophin pre-mRNA that is amenable for exon skipping. *See supra* § V.A.2.b; Dowdy Op. Rep. §§ V.C.4.a-b. Once this hot spot of exon 53 was identified, a POSA would have understood that ASOs having the claimed structural features would induce exon 53 skipping, similar to the four overlapping ASOs disclosed in the specification identifying the hot spot. *See* Wilton Tr. 55:4-10, 58:3-13; *see supra* § V.A.2.a. That numerous researchers thereafter made ASOs targeting this hot spot, and that all of the ASOs having the claimed structural features targeting the hot spot induced exon 53 skipping, further illustrates that the Wilton Patents resolved this unpredictability for the subject matter of the claimed invention. *See supra* § V.A.2.c.

267. Dr. Hastings' repeated reliance on a few paragraphs in the specification as the inventors' "admission" to the unpredictability is similarly deficient. *See* Hastings Rep. ¶116 (citing '851 Patent, 32:15-30). As discussed above, these paragraphs highlight the need for empirical identification of regions amenable to exon skipping, which the Wilton Patents describe for exon 53. *See supra* § V.A.3.b.iv; '851 Patent, 32:12-30; Fletcher Tr. 153:4-10 ("Q. And was that hot spot identified before these oligos in table 39 were synthesized? **A. I think, no, because we . . . determined [it] empirically. We couldn't have determined it without testing the oligos, and at the time there was no reliable computational testing tools that would determine which would and would not skip.**"). Notably, while Dr. Hastings cites to Dr. Wilton's testimony explaining what a term "empirical approach" means (Hastings Rep. ¶116 (citing Wilton Tr. 54:4-13)), she omits his subsequent testimony explaining the significance of the hot spot:

Q. What does "empirical approach" mean?

**A. A definition of empirical approach is do the experiment.**

Q. So it's -- you have to do the experiments and get the results and then draw the conclusions?

found in Ms. Adams' email. *See* WILTON0017692-95, -94 (email from Ms. Adams stating that "a few bases to the left or right can make a difference"). In fact, when Dr. Wilton in deposition was asked about the statement in Ms. Adams' email, he explained that this is context-dependent, for example, whether an ASO with a few bases changes still falls within the hot spot:

Q. Do you agree with her statement that a few bases to the left or right can make a difference?

**A. In some cases it can, but *there would still be exon skipping, and that is why we've defined the boundaries as plus 23 to 47, plus 39 to 69.***

Q. So your answer is yes, you agree that a few bases to the left or right can make a difference?

**A. It depends on the bases and the target.**

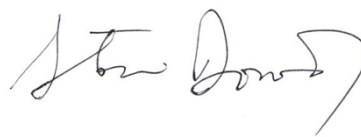
Wilton Tr. 185:23-186:9.

271. In sum, by focusing on the generalized unpredictability in the art instead of the claimed inventions, both Dr. Hastings and Dr. Wood fail to analyze the aspect at issue: predictability of exon 53 skipping within the hot spot set forth in the Wilton Patents. In view of the data reported in the Wilton Patents, a POSA would have expected that ASOs with the claimed structural features would induce exon 53 skipping. Real-world examples and the CERI Reports confirm this expectation.

**f. The Quantity of Experimentation Necessary**

**i. The Experimentation Needed for Practicing the Claimed Inventions, if Any, Would Have Been Routine**

272. Dr. Hastings contends that "[a]n enormous amount of experimentation would have been necessary to carry out the full scope of the claimed invention in 2005." Hastings Rep. ¶122. Specifically, Dr. Hastings argues that the work required to make and test "the full scope of the

A handwritten signature in black ink, appearing to read "Steven Dowdy", written over a horizontal line.

DATE: October 11, 2023

By: \_\_\_\_\_  
Steven F. Dowdy, Ph.D.

# Exhibit 3 to NS's MIL No. 1

**THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

NIPPON SHINYAKU CO., LTD.,

Plaintiff,

v.

SAREPTA THERAPEUTICS, INC.,

Defendant.

C.A. No. 21-1015 (GBW)

SAREPTA THERAPEUTICS, INC. and  
THE UNIVERSITY OF WESTERN  
AUSTRALIA,

Defendant/Counter-Plaintiffs,

v.

NIPPON SHINYAKU CO., LTD.  
and NS PHARMA, INC.

Plaintiff/Counter-Defendants.

**OPENING EXPERT REPORT OF STEVEN F. DOWDY, Ph.D.**

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100. By 2007, Dr. Wilton and colleagues had evaluated 470 ASOs targeting all exons of the dystrophin gene except exons 1 and 79. Wilton 2007, 1293 (“To date, we have evaluated 470 2OMeA[S]Os for targeted skipping of dystrophin exons 2-78, some of which have been described elsewhere.”) (citing, *inter alia*, Wilton PCT ’057). “Approximately two-thirds were found to induce detectable exon skipping,” wrote Dr. Wilton. *Id.* The tested exons were classified as Types 1, 2, 3, or 4, based on the level and manner in which the exon can be excised from the pre-mRNA. *Id.*, 1289. Exon 53 was classified as Type 1—among the most skip amenable exons (greater than 30% skipped product relative to the intact transcript). *Id.* Dr. Wilton’s contemporaneous presentations reiterate this viewpoint. *See, e.g.*, Wilton Presentation (WILTON0009927-948), WILTON0009948 (“53 ++++”).

101. My interpretation of the data in Wilton PCT ’057 is consistent with Dr. Wilton’s testimony in this litigation, explaining that he derived this hot spot from the data reported in the specification. Wilton Tr. 222:13-223:3, 223:6-21; *see also id.*, 22:1-22. The discovery of Dr. Wilton and colleagues provided confidence to other researchers in the field that other ASOs similarly targeting this hot spot of exon 53 would be expected to cause exon skipping, ultimately leading to the identification of at least two FDA approved drugs. *See infra* § VI.

**c. After June 2005: AVI BioPharma, Royal Holloway, and University of Western Australia**

102. The hot spot identified in 2005 by Dr. Wilton and colleagues continued to be targeted and refined for the next several years by Dr. Wilton’s group and other independent researchers. By August 31, 2011, it was appreciated that within that hot spot, the narrow (+30+65) region of human exon 53 is particularly amenable for exon skipping.

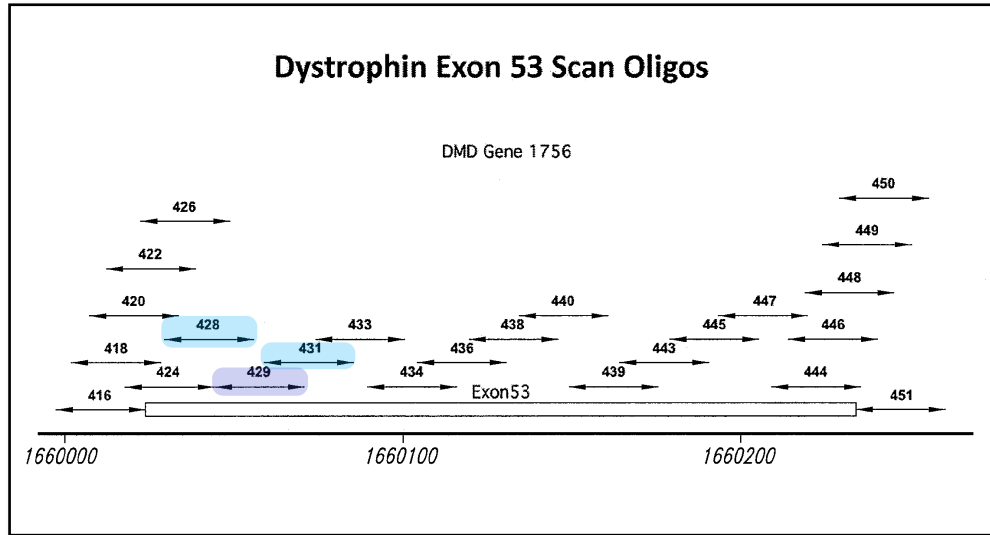
103. Researchers at AVI BioPharma (now known as Sarepta Therapeutics), including Drs. Peter Sazani and Ryszard Kole, further confirmed the exon 53 hot spot. In International

Patent Publication No. WO 2010/048586 (“Sazani PCT ’586”), Drs. Sazani and Kole described a series of peptide-conjugated PMOs targeting human exon 53 that were made and tested in human rhabdomyosarcoma (“RD”) cells and primary human skeletal muscle cells. Sazani PCT ’586, 75-76. The peptide-conjugated PMOs tested in the Sazani PCT are PMOs conjugated to a small peptide that facilitates cellular uptake, often referred to as a “PPMO.” *Id.*; Nakamura 2009, Figure 1. Among the tested PPMOs, SEQ ID NOs: 428, 429, and 431 were identified as effective in inducing exon skipping, with SEQ ID NO: 429 shown to be particularly effective. Sazani PCT ’586, 75-76. As shown below, SEQ ID NOs: 428, 429, and 431 are complementary to the (+8+32), (+23+47), and (+38+62) regions of human exon 53, respectively.

Comparison Between the Nucleotide Sequence of Human Exon 53 and SEQ ID NOs: 428, 429, 431	
Exon 53	5'-uugaaagaau ucagaaucag ugggaugaag uacaagaaca ccuucagaac cggaggcaac 60
SEQ ID NO: 428	3'-TTA AGTCTTAGTC ACCCTACTTC AT-5'
SEQ ID NO: 429	3'-CCTACTTC ATGTTCTTGT GGAAGTC-5'
SEQ ID NO: 431	3'-TGT GGAAGTCTTG GCCTCCGTTG
	aguugaauga aauguuaaag gauucaacac aauggcugga agcuaaggaa gaagcugagc 120
	TC-5'
	aggucuuagg acaggccaga gccaaagcuug agucauggaa ggaggguccc uauacaguag 180
	augcaaucca aaagaaaatc acagaaacca ag-3' 212

**Table 4.** SEQ ID NOs: 428, 429, and 431 from Sazani PCT ’586

104. I have also annotated Figure 4A of Sazani PCT ’586 below, identifying the three ASOs that Drs. Sazani and Kole identified as effective in inducing exon skipping. As Drs. Sazani and Kole further explained, the most effective one, SEQ ID NO: 429, corresponded to H53A(+23+47) reported by Dr. Wilton and colleagues in Wilton PCT ’057. Sazani PCT ’586, 75-76. Another effective ASO, SEQ ID NO: 431, targeted the (+38+62) region of human exon 53. *Id.* In other words, Drs. Sazani and Kole independently confirmed the hot spot identified by Dr. Wilton and his coinventors, including in particular the region targeted by H53A(+23+47). *See* Wilton PCT ’057, Table 39.



**Figure 17.** Annotated Figure 4A of Sazani PCT '586

105. A separate patent application from Dr. Wilton's group, International Patent Application No. PCT/AU2011/001520, further confirmed his group's earlier discovery of the exon 53 hot spot. The application was published as International Patent Publication No. WO 2011/057350 ("Wilton PCT '350") in May 2011. Here, Dr. Wilton and colleagues disclosed more than 20 ASOs targeting human exon 53. Wilton PCT '350, 58-59. These were made as 2'OMePS oligonucleotides and tested in primary human myotubes. *Id.*, 33-35. As shown in Table 43 of the Wilton PCT '350, many of those ASOs fell within or overlapped with the previously-identified hot spot and induced exon skipping. *Id.*, Table 43. In contrast, those that did not overlap with the hot spot did not induce skipping, reiterating the importance of targeting the hot spot in inducing exon 53 skipping. *See id.*

106. Drs. George Dickson and Linda Popplewell at Royal Holloway University of London both confirmed the exon 53 hot spot and identified a narrower region within that hot spot that is particularly amenable for exon skipping. This work was documented in two articles published in 2009 and 2010, respectively, and a patent publication published in 2010. *See*

Popplewell 2009; Popplewell 2010; U.S. Patent Publication No. 2010/0168212 (“Popplewell US ’212”).

107. In 2009, Drs. Dickson and Popplewell designed a set of 66 PMOs targeting human exons 44, 45, 46, 51, and 53 of the dystrophin pre-mRNA. Popplewell 2009, 554. The design strategy used a combination of available prediction tools and empirical evaluation. *Id.*, 554-55; Popplewell US ’212, [0059]. For example, using tools available then, Drs. Dickson and Popplewell identified potential regions within exon 53 involved in the splicing process. Popplewell 2009, 554-55; Popplewell US ’212, [0059]. These regions were then compared against regions within exon 53 that were physically accessible in an *in vitro* hybridization assay. Popplewell 2009, 554-55; Popplewell US ’212, [0059]. The overlapping regions between the two were then interrogated with an array of PMOs that are 25 bases in length. Popplewell 2009, 554-55; Popplewell US ’212, [0059]. Each PMO was tested in primary human myotubes. Popplewell 2009, 554-55; Popplewell US ’212, [0060].

108. Consistent with the earlier identification of the hotspot, of the 25-mer PMOs evaluated, four PMOs were shown to cause exon 53 skipping, PMO-A, -B, -C, and -D, complementary to the (+35+59), (+38+62), (+41+65), and (+44+68) regions of human exon 53, respectively. Popplewell US ’212, [0085]. Based on these active 25-mer PMOs, Drs. Dickson and Popplewell then designed a “3 nt-stepped array” of PMOs that are 30 bases in length (i.e., staggered by 3 bases) to focus on this active region. *Id.*, [0086]. Collectively, these 30-mer PMOs were complementary to the (+30+74) region of human exon 53. *Id.* Among them were PMO-G, -H, -I, -J, and -K, which showed detectable skipping in primary human myotubes and were complementary to the (+30+59), (+33+62), (+36+65), (+39+68), and (+42+71) regions of human exon 53, respectively. *Id.*; *see also* Popplewell 2010, Table 1(a). They concluded that “the



2'OMePS A[S]Os displaying the highest bioactivity in the work of Aartsma-Rus *et al.* and Wilton *et al.* show some degree of overlap with the hybridization peaks that we have defined here for exons 45, 46, and 53.” Popplewell 2009, 558-59.

109. In a subsequent paper published in 2010, Drs. Dickson and Popplewell evaluated those previously identified active PMOs in muscle cells derived from a DMD Patient and in mice carrying the human dystrophin gene. Popplewell 2010, Abstract. Dr. Wilton’s sequence, H53A(+39+69), was also evaluated as a comparator. *See id.*, Table 1(a), 103. All of those PMOs were effective in causing skipping in cells. *Id.*, Figure 1(b). Among them, the PMOs targeting the (+30+65) region of human exon 53 were particularly effective, leading the authors to recommend that “PMOs targeting sequence +30+65 of exon 53 of the *DMD* gene [are] worthy of consideration for any upcoming clinical trial.” *Id.*, 109; *see also id.*, 103 (“Collectively, this work reports a number of PMOs able to produce targeted skipping of exon 53 to levels that would suggest them worthy of consideration for upcoming PMO clinical trials.”); *see infra* § X.B.1.a.

**d. Exemplary ASOs Targeting Exon 53 Known as of August 31, 2011**

110. As summarized above, by August 31, 2011, the hot spot within exon 53 had been identified and repeatedly verified. In **Figure 18** below, I have summarized the ASOs published as of August 31, 2011 that are within or near to the hot spot described in Wilton PCT ’057 (shaded with pink) that were reported to induce exon 53 skipping. The narrower region that Drs. Dickson and Popplewell further described in 2010 is shaded with purple. Of note, I excluded the ENA ASOs that Dr. Matsuo and colleagues published in 2004, as interpretation of that data is difficult for the reasons discussed above.

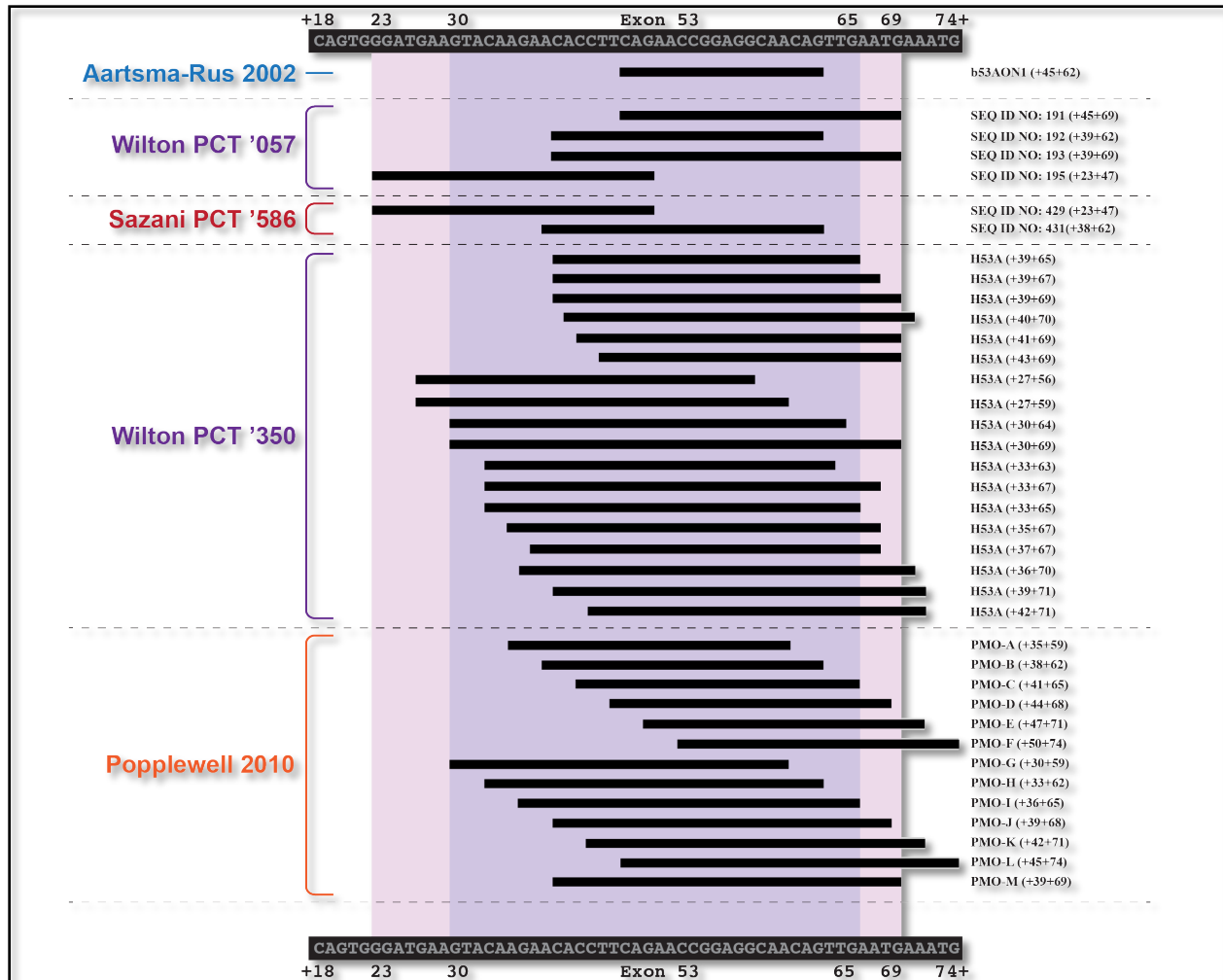


Figure 18. Exon 53 Targeting ASOs as of August 31, 2011

## 5. Methods for Making and Testing DMD Targeting ASOs

### a. PMO Synthesis Methods

111. As Dr. Pentelute explains, methods of synthesizing PMOs had been known since the early 1990s. Pentelute Op. Rep. §VI.B; *see also* Watanabe Ex. 5(A) (listing of various publications from the 1990s disclosing PMO synthesis methods). Like other polymeric molecules, it was known that PMOs could be made through a repeated cycle of: (1) deprotecting a growing polymeric chain and (2) coupling a monomeric compound to the deprotected polymeric chain. *See, e.g.*, Summerton 1997, Figure 5; Summerton 2003, Figure 4; Gene Tools; *see also* Watanabe

Tools, 5; Popplewell US '212, Figure 3 (predicted secondary structure of exon 53 of the human dystrophin pre-mRNA).

**b. Considerations in Designing Therapeutic ASOs**

116. As of August 2011, researchers considered several factors in designing therapeutic ASOs. One factor was the empirical identification of a hot spot in a targeted exon, shown experimentally to induce exon skipping by overlapping ASOs. While certain *in silico* prediction tools were available, they were unreliable. *See, e.g.*, Harding 2007, 164 (recommending that “identification of amenable sites should be carried out empirically”). Once empirically identified, researchers often focused on identifying additional ASOs within or near the identified hot spot. *See, e.g.*, Popplewell 2010, 108 (“The data presented here would indicate that PMOs targeting within the sequence +30+65 of exon 53 . . . produce levels of exon skipping that may be considered effective (over 50% exon skipping). There remains however the possibility that a stepped base-by-base screening of ASOs across the entirety of exon 53 and some indeterminant distance into the flanking intronic sequences might reveal an ASO with a better dose-response and longevity of action profile.”).

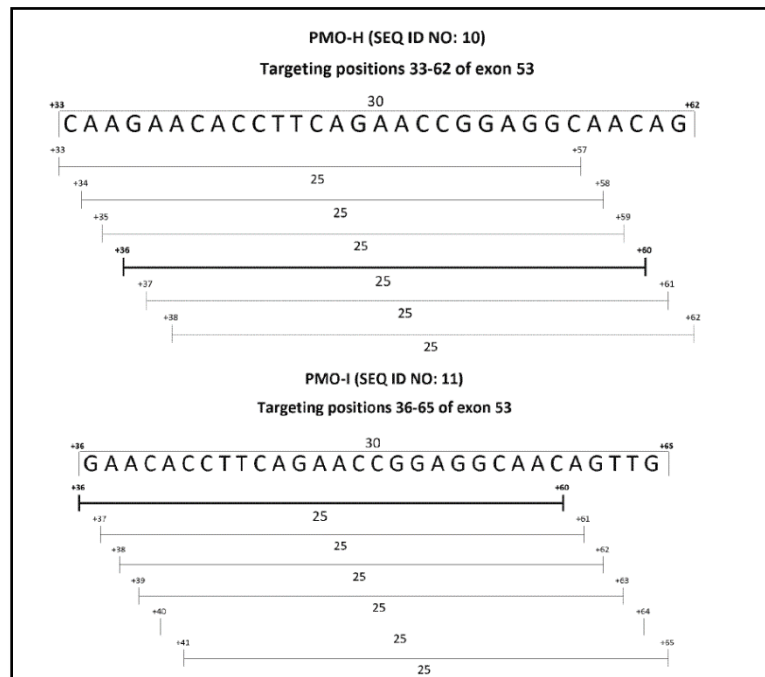
117. The choice of backbone chemistry was another consideration. As discussed above, the two prominent chemistry backbones as of August 2011 were 2'OMePS and PMO. *See supra* § V.C.3. For example, the PMO backbone was known to confer various biological and drugability benefits, suitable for a therapeutic drug. Hoffman 2011, 16. The 2'OMePS backbone was widely accessible among researchers due to ease of manufacture. Nakamura 2009, 495-96. By August 2011, the skipping efficiency between 2'OMePS and PMO directed to the same target region was generally correlative, and as such, researchers recommended using 2'OMePS as an initial screening tool with the ultimate goal of developing a corresponding PMO as a therapeutic drug.

VYDS-0201525. The project involved a multicenter collaboration among Sarepta, University College London (“UCL”), and Royal Holloway – University of London (“RHUL”). *Id.*

130. The report, signed by Dr. Diane Frank in 2014, provides an overview of exon 53 screens reported in scientific publications and the patent literature. *Id.*, SRPT-VYDS-0201529. Table 1 of the report lists several patent publications, including specifically those discussed above. *See id.* (Table listing Wilton PCT ’057, Sazani PCT ’586, Wilton PCT ’350, and Popplewell US ’212); *see supra* §§ V.C.4.b-d. The report explains that Wilton PCT ’057 “identified an effective target region of +23 to +69 relative to the splice acceptor site.” Sequence Selection Report, SRPT-VYDS-0201529. The report further explains that this effective target region was independently confirmed by work done at Sarepta (as reported in Sazani PCT ’586) and RHUL (as reported in Popplewell US ’212). *Id.*, SRPT-VYDS-0201529-30. Eight PMOS, all of which were “internal to Exon 53 and fell within the previously identified effective region (+23+69),” were proposed by Sarepta and RHUL. *Id.*, SRPT-VYDS-0201531-32. Those PMOs ranged from 25 to 31 bases in length. *Id.*, Table 2.

131. Each of these eight PMOs were tested in blinded studies by each of RHUL, UCL, and Sarepta. *Id.*, SRPT-VYDS-0201548-557. At RHUL, these PMOs were evaluated in: (1) two types of cells derived from patients with DMD and (2) normal human skeletal muscle primary cells at varying concentrations. *Id.*, SRPT-VYDS-0201548-554. All of the PMOs induced strong skipping in a variety of tested conditions. In the first type of cells derived from patients with DMD, “[s]kipping was evident with all eight PMOs, and at all concentrations tested.” *Id.*, SRPT-VYDS-0201548-550. In the second type of cells derived from patients with DMD, “very high levels of exon skipping with all PMOs at all concentrations tested” were observed, ranging from “85% to 96% skipping.” SRPT-VYDS-0201553. Similar results were obtained from normal

overlapping antisense oligomers that target exon 53,” including “25 base antisense oligomers targeting positions 36-60 of exon 53, as recited in claim 1.” Notice of Opposition 2, SRPT-VYDS-0223977-79.



Notice of Opposition 2, SRPT-VYDS-0223979.

415. Before an oral proceeding was held, I understand that NS conceded, stating that it “no longer approves of the text in which the above-mentioned patent [EP ’211] was granted and will not submit an amended text.” NS Notice of Abandonment, SRPT-VYDS-0226797.

416. The novelty arguments from James Poole Limited and NS’s subsequent decision to abandon its European Patent further support my opinion that the asserted claims of the ’361, ’092, ’461, ’106, ’741, and ’217 Patents are anticipated and therefore are invalid.

**B. All of the Asserted Claims of the NS Patents Would Have Been Obvious Over the Combination of Popplewell 2010 and Sazani 2010, Further in View of Reeves PCT ’471**

417. As of August 31, 2011, exon skipping ASOs had emerged as a promising strategy for treating DMD. Exon 51 targeting ASOs were already in clinical trials, and researchers were

actively working on developing ASOs targeting other exons. This included exon 53, which was believed to potentially treat one of the largest populations of DMD patients. By then, numerous ASOs targeting exon 53 had been made and tested by multiple independent research groups focusing on the “hot spot” region identified by Dr. Wilton and colleagues. Indeed, by August 2011, a narrower (+30+65) region of human exon 53 had been defined by work from Drs. Popplewell and Dickson, as published in Popplewell 2010. Popplewell 2010 expressly encouraged others to investigate this region, recommending that “PMOs targeting sequence +30+65 of exon 53 of the DMD gene [are] worthy of consideration for any upcoming clinical trial.” Popplewell 2010, 109.

418. A POSA would have been motivated to use an array of ASOs having the standard length of 25 bases to further explore the narrow (+30+65) region taught in Popplewell 2010, including an ASO complementary to the (+36+60) region of human exon 53—the same target region now claimed by the NS Patents. A POSA also would have been motivated to move the target regions for the viable clinical candidates taught in Popplewell 2010, which also would have identified an ASO complementary to the (+36+60) region of human exon 53. A POSA would have reasonably expected that any such ASOs, including one complementary to the (+36+60) region, would successfully cause exon skipping. Indeed, *all* of the 25-mer PMOs reported in Popplewell 2010 that fall within the narrow (+30+65) region caused exon skipping. The expectation of success would have been buttressed by other exon 53 targeting ASOs developed by August 2011, all of which were both (1) complementary to regions overlapping this narrowed region and (2) reported to cause exon skipping. Understanding the relationship between exon skipping and DMD pathophysiology, a POSA would have reasonably expected that an ASO

targeting the (+36+60) region of human exon 53 could successfully treat DMD in patients amenable for exon 53 skipping.

419. A POSA interested in developing a promising therapeutic ASO would have been motivated to use a PMO with a TEG modification based on Sazani 2010. As of August 2011, the therapeutic potential of a PMO with a 5'-TEG modification had been extensively investigated. For example, nonhuman primate studies reported in Sazani 2010 demonstrated the superior therapeutic window of a PMO with a 5'-TEG modification. *See* Sazani 2010, Abstract. The prior art also recognized numerous biological and drugability benefits for such PMO ASOs, including a lack of immunogenicity and improved stability and solubility. A POSA would have had a reasonable expectation of successfully synthesizing a PMO with a TEG modification using synthesis methods known for decades, including one developed by AVI BioPharma and reported in Reeves PCT '471.

420. For these reasons and additional reasons discussed below, it is my opinion that the asserted claims of the NS Patents would have been obvious to a POSA as of August 31, 2011. That an independent group identified the claimed PMO just five months after the August 31, 2011 filing date of the NS Patents further illustrates obviousness of the claimed subject matter. *See de Visser* US '354. In my opinion, all of the asserted claims of the NS Patents would have been obvious as of August 2011.<sup>20</sup>

### **1. Review of Prior Art**

421. As further discussed below, the asserted claims of the '361, '092, '461, '106, '741, and '217 Patents would have been obvious over Popplewell 2010 and Sazani 2010, and optionally

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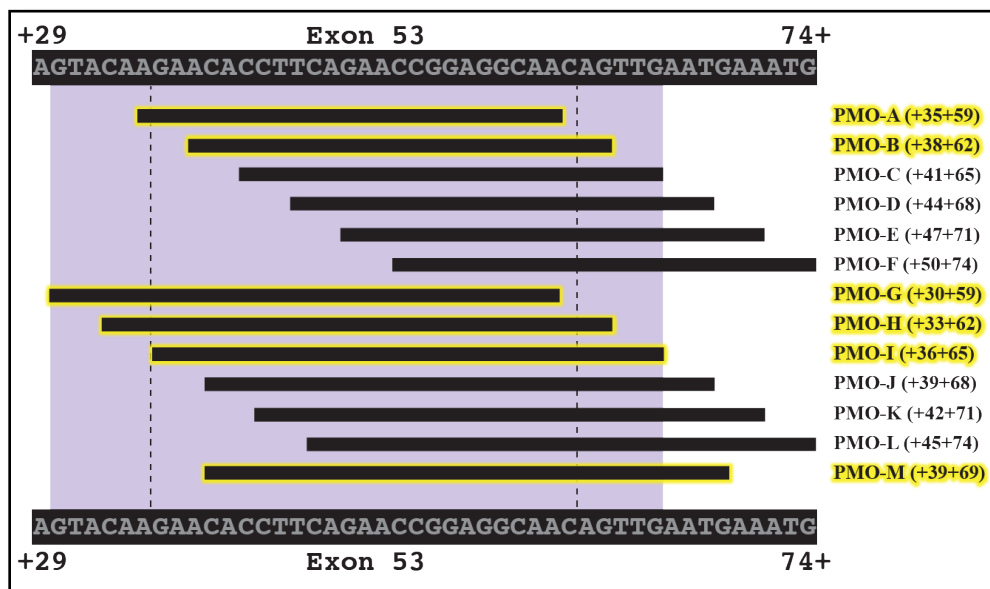
<sup>20</sup> From my review of the documents available to me today, my opinion is that the claimed ASOs of the NS Patents do not exhibit superior skipping activity versus certain PMOs reported in the prior art. *See infra* § XI. I understand that NS has the burden of demonstrating objective indicia of non-obviousness. I understand that I will have the opportunity to respond to any objective indicia of non-obviousness raised by NS's experts.

further in view of Reeves PCT '471. The asserted claims of the '322 Patent would have been obvious over Popplewell 2010, Sazani 2010, and Reeves PCT '471. Each of the references is summarized below.

**a. Popplewell 2010**

422. According to the journal website, Popplewell 2010 was published as a part of the February 2010 volume of *Neuromuscular Disorders*. See Popplewell 2010 Journal Website. This is more than one year before the effective filing date of each of the NS Patents—August 31, 2011. See *supra* § VIII.C. I understand that Popplewell 2010 therefore qualifies as prior art against the NS Patents for at least this reason.

423. Popplewell 2010 disclosed “the methodical, cooperative comparison, in vitro (in DMD cells) and in vivo (in a transgenic mouse expression human dystrophin), of 24 A[S]Os of the [PMO] chemistry designed to target exon 53 of the *DMD* gene.” Popplewell 2010, Abstract. Among them, thirteen PMOs, labeled PMO-A through -M, were evaluated in cells derived from DMD patients. These PMOs targeted the (+29+74) region of human exon 53.



**Figure 31.** 13 PMOs Tested in Popplewell 2010  
(Type 1 PMOs Highlighted in Yellow)



444. For at least these reasons, a POSA would have been motivated to make ASOs targeting human exon 53 and to administer them to DMD patients intravenously in view of Popplewell 2010 and Sazani 2010.

**b. A POSA Would Have Been Motivated to Make and Use a 25-Mer PMO that Is 100% Complementary to the (+36+60) Region of Human Exon 53**

445. Claim 1 of the '217 Patent recites a “[PMO] that is 100% complementary to the 36th to the 60th nucleotides from the 5' end of the 53rd exon in a human dystrophin pre-mRNA,” which consists of a nucleotide sequence corresponding to SEQ ID NO :1.” Claim 1 further recites a chemical structure for the PMO monomers and a 5'-TEG modification. For at least reasons discussed below, a POSA would have been motivated to make and use the claimed PMO in view of Popplewell 2010 and Sazani 2010.

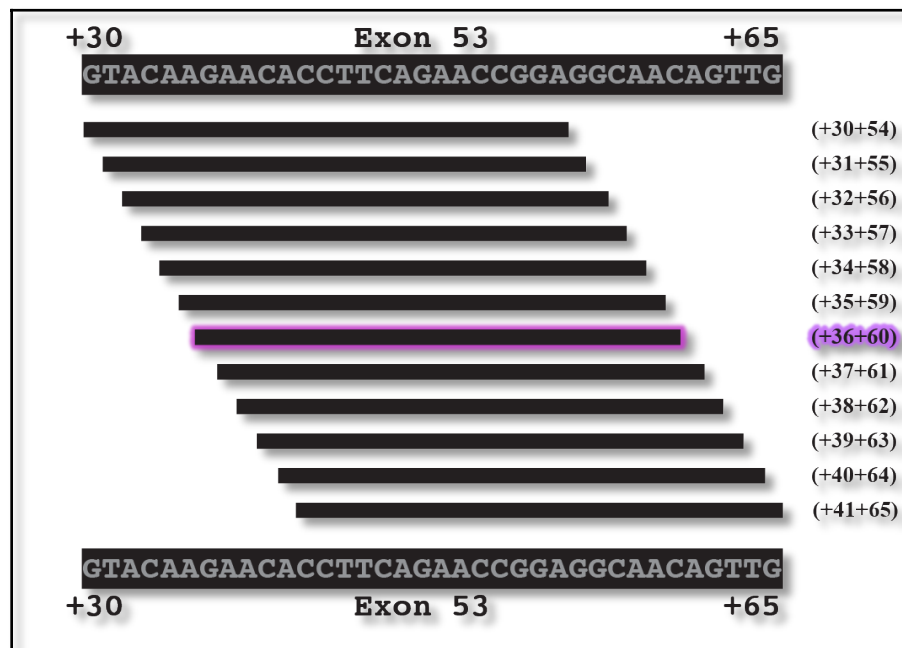
**i. A POSA Would Have Been Motivated to Investigate the Narrow (+30+65) Region of Human Exon 53 Taught in Popplewell 2010**

446. Although human exon 53 is a relatively lengthy exon consisting of 212 bases (flanked by two lengthier introns), a POSA would have known which region to investigate—the exon 53 hot spot identified by Dr. Wilton. *See supra* § V.C.4.b; Wilton PCT '057, Table 39, Figure 22. Indeed, researchers had subsequently verified and refined this discovery, describing multiple overlapping ASOs targeting this region that caused exon skipping under a variety of experimental conditions. *E.g.*, Sazani PCT '586, 75-76; Wilton PCT '350, 58-59; Popplewell US '212, [0084]-[0098], Table 4; *see supra* §§ V.C.4.c-d.

447. Within this hot spot, a POSA would have been motivated to focus on the (+30+65) region of human exon 53 recommended in Popplewell 2010. *See supra* § X.B.1.a. As discussed above, Popplewell 2010 stated that “the superiority of the PMOs targeting the sequence +30+65 (i.e. PMOs-A, -B, -G, and -H) is strongly indicated.” Popplewell 2010, 109. Popplewell 2010

in transgenic mice); *see also supra* § V.C.3 (clinical trial studies reporting exon skipping caused by AVI-4658).

458. Considering these factors together, a POSA conducting a one-base microwalk experiment focusing on the (+30+65) region of human exon 53 would have been motivated to make an ASO that is 25 bases in length and 100% complementary to the (+36+60) region of exon 53 (highlighted in purple).



**Figure 39.** 25-mer Microwalk of the (+30+65) Region of Human Exon 53

459. As shown in **Figure 40**, NS's internal documents confirm the routine exploration of the exon 53 hotspot via microwalk as of August 2011. For example, in 2009, two of the named inventors of the NS Patents, Mr. Watanabe and Mr. Satou, evaluated an array of 25-mer ASOs covering the entirety of human exon 53. Within a matter of weeks, they had completed those experiments, reconfirming Dr. Wilton's identification of the exon 53 hotspot. *See* Watanabe Ex. 18(A), NS00061819 (planning to "[REDACTED]").

[REDACTED]

460. The NS researchers then “[REDACTED]

[REDACTED]. See Watanabe Ex. 18(A), NS00061819; Watanabe Tr. 109:4-8 (Mr. Watanabe confirming that Watanabe Ex. 18(A) was prepared on or about July 8<sup>th</sup> 2009). Again, within a matter of weeks, several arrays of 25-mer ASOs focusing on different sites within human exon 53 were constructed and evaluated, including an array of 25-mers that were staggered by one base and focused on the (+29+65) region of human exon 53:

[REDACTED]

targeting ASOs, and GlaxoSmithKline had publicly announced its investment into a development program for exon 53 skipping ASOs. *See supra* § X.B.2.a.

492. Popplewell 2010 and Sazani 2010 identified a finite number of solutions to this problem. *See supra* § X.B.2.b. Popplewell 2010 reported that a narrow (+30+65) region of human exon 53 within the hot spot identified by Dr. Wilton and colleagues was particularly amenable to exon skipping. *See supra* §§ X.B.1.a, X.B.2.b.i. Popplewell 2010 expressly recommended that “PMOs targeting sequence +30+65 of exon 53 of the DMD gene [are] worthy of consideration for any upcoming clinical trial,” and identified PMO-A targeting (+39+59) as a viable clinical candidate. *See supra* §§ X.B.1.a, X.B.2.b.i. Using an array of 25-mer ASOs, which was the standard length of ASOs in August 2011, a POSA would have immediately envisaged a finite number of ASOs, including the claimed 25-mer ASO that is 100% complementary to the (+36+60) region of human exon 53. *See supra* § X.B.2.b.ii. Even if a POSA had been interested in additional lengths (e.g., from 25 bases up to 30 bases in length), a POSA would have immediately envisaged a finite number of ASOs, including the claimed 25-mer ASO that is 100% complementary to the (+36+60) region of human exon 53. *Id.* All of these ASO options squarely fall within the hot spot of human exon 53 identified by Dr. Wilton in 2005 and refined and verified by other researchers. *Id.*; *see supra* § X.B.2.d. Consequently, there were a finite number of identified, predictable solutions in the prior art that would have been expected to cause exon 53 skipping and treat DMD. *See supra* §§ X.B.2.d-e.

493. Sazani 2010 additionally offered a finite number of solutions. As of August 2011, it was well understood that ASOs need to be chemically modified to be therapeutically viable. *See supra* § V.C.3. By August 2011, there were a limited number of chemical backbone modifications used for ASOs in clinical trials for treating DMD, namely 2'OMePS and PMO backbone




A handwritten signature in black ink, appearing to read "Steven Dowdy", written over a horizontal line.

DATE: September 7, 2023

By: \_\_\_\_\_  
Steven F. Dowdy, Ph.D.

IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE

NIPPON SHINYAKU CO., LTD., )  
 )  
Plaintiff, )  
 )  
v. ) C.A. No. 21-1015 (JLH)  
 )  
SAREPTA THERAPEUTICS, INC., )   
 )  
Defendant. )

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SAREPTA THERAPEUTICS, INC. and THE )  
UNIVERSITY OF WESTERN AUSTRALIA, )  
 )  
Defendant/Counter-Plaintiffs, )  
 )  
v. )  
 )  
NIPPON SHINYAKU CO., LTD. )  
and NS PHARMA, INC. )  
 )  
Plaintiff/Counter-Defendants. )

**SAREPTA THERAPEUTICS, INC. AND THE UNIVERSITY OF WESTERN  
AUSTRALIA’S OPPOSITION TO NIPPON SHINYAKU CO., LTD. AND NS PHARMA,  
INC.’S MOTION IN LIMINE NO. 1 TO PRECLUDE IMPROPER RELIANCE ON  
POST-PRIORITY DATE EVIDENCE TO SUPPORT THE UWA PATENTS’ VALIDITY**

Sarepta's<sup>1</sup> post-priority evidence is entirely appropriate. As a threshold matter, this evidence is highly probative of enablement, because it shows that the field routinely obtained the claimed exon 53-skipping ASOs by following the teachings in the patents. Indeed, *none* of NS's cases precludes post-priority evidence of enablement; to the contrary, both the Federal Circuit and this Court have endorsed reliance on such evidence. In addition, Sarepta's evidence is highly probative of written description, because it describes and illuminates the state of the art as of the priority date. This contrasts with the fact-specific cases cited by NS, where a patentee's post-priority evidence was irrelevant to written description (as opposed to enablement). Rather than identifying new ASO sequences or properties, Sarepta's evidence confirms the patent's teachings, *i.e.*, that the claimed ASOs induce exon 53-skipping. Worse yet, NS wants to have it both ways, itself relying on post-priority testing as evidence of alleged invalidity, while simultaneously trying to prevent Sarepta from relying on any post-priority evidence – including the portions of *NS's own testing* that prove the claimed ASOs work. NS Op. Br. at Appendix A (643, 644 and 661 CERI Reports). The law does not support such a double standard, which would be manifestly unfair to Sarepta.

Starting with enablement, the Federal Circuit has repeatedly allowed the same sort of post-priority evidence that Sarepta intends to rely on here. *See, e.g., Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1336 (Fed. Cir. 2003) (“[N]umerous post-filing publications . . . demonstrated the extent of the enabling disclosure.”); *In re Brana*, 51 F.3d 1560, 1567 n.19 (Fed. Cir. 1995) (post-priority declaration listing test results of the claimed compounds “goes to prove that the disclosure was in fact enabling when filed”); *Gould v. Quigg*, 822 F.2d 1074, 1078 (Fed. Cir. 1987) (allowing “later dated publication . . . offered as evidence of the level of ordinary skill

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<sup>1</sup> For purposes of this brief, “Sarepta” refers to Sarepta and/or UWA.

in the art at the time of the application and as evidence that the disclosed device would have been operative”); *see also In re Hogan*, 559 F.2d 595, 605 (C.C.P.A. 1977) (noting “approved use of later publications as evidence of the state of art existing on the filing date of an application.”). This Court has likewise allowed such evidence. *See Amgen Inc. v. Sanofi*, Civ. No. 14-1317-RGA, 2019 WL 11071409, at \*2 (D. Del. Feb. 14, 2019) (*Amgen II*) (allowing post-priority evidence that “confirms enablement of [patentee’s] disclosed roadmap”). Sarepta’s post-priority evidence is all the more relevant because of NS’s own self-serving reliance on its post-priority testing and other post-priority evidence. Exs. A–E.

Sarepta’s evidence is equally probative of written description. While NS cites a handful of cases in which *certain types* of patentee post-priority evidence were found to be irrelevant to written description (*not* enablement), those cases are factually distinguishable.<sup>2</sup> NS Op. Br. at 1–2. NS mischaracterizes those cases as involving the same sort of “confirmatory post-priority date evidence” that Sarepta relies on. *Id.* Those cases addressed *new discoveries and developments* – not confirmatory evidence. Here, there are a finite number of exon 53-skipping ASOs that are already structurally defined in the patent claims. Sarepta is not relying on post-priority evidence to identify new ASO sequences or new properties of the claimed ASOs. Instead, Sarepta relies on such evidence for the narrow, proper purpose of confirming that the claimed ASOs induce exon

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<sup>2</sup> *Juno Therapeutics, Inc. v. Kite Pharma, Inc.*, 10 F.4th 1330, 1340–41 (Fed. Cir. 2021) (very few known examples of claimed CD19-specific single-chain antibody variable fragments (scFvs) as of the priority date; later development irrelevant to written description); *Biogen Int’l GMBH v. Mylan Pharms. Inc.*, 18 F.4th 1333, 1343 (Fed. Cir. 2021) (claimed dose not sufficiently described in specification; “[t]hat Biogen later established the therapeutic efficacy of DMF480 is of no import to the written description analysis”); *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1355–58 (Fed. Cir. 2010) (specification did not adequately describe “molecules that Ariad admits are necessary to perform the [claimed] methods”; post-priority evidence did not establish possession); *MorphoSys AG v. Janssen Biotech, Inc.*, 358 F. Supp. 3d 354, 367 (D. Del. 2019) (post-priority evidence of common structural features not relevant to written description because it relied on an antibody’s sequence and binding partner, which were not known as of the priority date).



53-skipping. *See Amgen II*, 2019 WL 11071409, at \*2–3 (allowing post-priority evidence that “illuminates the state of the art at the priority date”). As in the enablement context, Sarepta’s post-priority evidence is all the more relevant in view of the competing NS post-priority evidence.

NS is also wrong that only patent challengers (in this instance, NS) – and not patentees (Sarepta) – are permitted to rely on post-priority evidence. NS Op. Br. at 1 (citing *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1375 (Fed. Cir. 2017) (*Amgen I*)). None of the cases that NS cites hold that a patentee’s post-priority evidence is de facto inadmissible. *Id.* at 1–2. As explained *supra*, both the Federal Circuit and this Court permit a patentee to rely on post-priority evidence of validity, when relevant.

NS’s remaining complaints are similarly misplaced. NS argues that Sarepta’s evidence “is *not* commensurate with the ‘full scope’” of the claims. NS Op. Br. at 3. But that is an issue for cross-examination and goes to the weight of the evidence, not its admissibility. NS also argues that “Sarepta and UWA should not be allowed to backdoor these arguments in connection with its obviousness arguments against the NS Patents.” *Id.* But, it is NS that appears to be seeking a “backdoor” exclusion of otherwise admissible *pre-priority* evidence of obviousness of NS’s patents, which have a much later priority date (August 2011) than Sarepta’s patents (June 2005).

In sum, Sarepta’s reliance on its post-priority evidence is highly relevant to the § 112 issues in this case. Moreover, NS should not be permitted to selectively rely on post-priority evidence of allegedly inoperative embodiments, while Sarepta is unable to refute NS’s evidence with its own such evidence. For all these reasons, the Court should deny NS’s motion in its entirety.

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April 25, 2024

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**CERTIFICATE OF SERVICE**

I hereby certify that on April 25, 2024, copies of the foregoing were caused to be served upon the following in the manner indicated:

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/s/ Megan E. Dellinger

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# Exhibit A



Study Number	936-21-M-0643
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## FINAL REPORT

The assessment of exon skipping activities by 2'-OMe-S-RNA in myotube using Lipofectin as a transfection reagent.

May 2023

Chemicals assessment and research center  
Chemicals Evaluation and Research Institute, Japan (CERI)

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1. TITLE



2. STUDY NUMBER

936-21-M-0643

3. OBJECTIVE

To assess exon skipping activities of four different 2'-OMe-S-RNA (ASO) or mixture of ASO in myotube.

4. TESTING FACILITY

Name: Chemicals Assessment and Research Center, Chemicals Evaluation and Research Institute, Japan (CERI)  
Address: 1600, Shimotakano, Sugito-machi, Kitakatsushika-gun, Saitama 345-0043, Japan

5. PERIOD OF STUDY

Study initiation date.:	February 2, 2022
Experimental starting date.:	February 3, 2022
Experimental completion date.:	April 14, 2022
Study completion date.:	May 17, 2023

6. MATERIALS AND METHODS

6.1 Test substance and vehicle

6.1.1 Test substances

The test substances used in this study were listed in Table 1.

Table 1 Information of test substances

Test substance ID	
Test substance name	
Sequence (5' – 3')	
Binding position	
Lot number	
Physicochemical properties	
Appearance in ordinal temperature	
Molecular weight	
Purity	
Solubility	
Storage condition of test substance	
Storage condition of test compound solution	

\*1: -70°C or less, \*2: -20 °C or less

Gloves, a mask and a lab coat were worn when handling these substances.



6.1.2 Vehicle control substance

(1) Name

Water for injection

(2) Grade

Japanese Pharmacopoeia

(3) Lot number

K9K87

(4) Manufacturer

Otsuka Pharmaceutical Factory

(5) Storage condition

Vehicle was stored at room temperature.

(6) Handling precautions

Gloves, a mask, and a lab coat were worn when handling.

6.2 Cells

Name: [REDACTED]

Supplier: [REDACTED]  
[REDACTED]

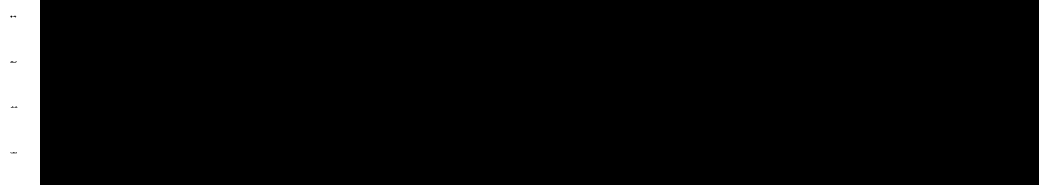
Lot number: [REDACTED]

6.3 Reagents, equipments and softwares

(1) Reagents

- PBS (-) powder (Thermo Fisher Scientific)
- SkGM™-2 BulletKit™ (Lonza)
- Opti-MEM (Thermo Fisher Scientific)
- Trypsin (Thermo Fisher Scientific)
- Dulbecco's Modified Eagle Medium: F12 (DMEM: F12) (Lonza)
- Horse serum (Thermo Fisher Scientific)
- Trypan Blue Stain 0.4% (Thermo Fisher Scientific)
- Lipofectin (Thermo Fisher Scientific)
- Trizol (Thermo Fisher Scientific)
- 2-Propanol (Molecular Biology, FUJIFILM Wako Pure Chemical)
- Ethanol (Guaranteed Reagent, FUJIFILM Wako Pure Chemical)
- Chloroform (Guaranteed Reagent, FUJIFILM Wako Pure Chemical)
- Water for injection (Grade: Japanese Pharmacopoeia, Otsuka Pharmaceutical Factory)
- QIAshredder (QIAGEN)

- RNeasy PLUS Micro Kit (QIAGEN)
- High Sensitivity RNA ScreenTape (Agilent)
- High Sensitivity RNA Sample Buffer (Agilent)
- High Sensitivity RNA Ladder (Agilent)
- Nuclease Free Water (Thermo Fisher Scientific)
- Titan One-tube RT-PCR system (Sigma-Aldrich)



- Tth DNA Polymerase (Sigma-Aldrich)
- PCR Nucleotide Mix (Sigma-Aldrich)
- High SensitivityD1000 ScreenTape (Agilent)
- High Sensitivity D1000 Reagents (Agilent)
- High Sensitivity D1000 Ladder (Agilent)
- TE Buffer, 1X (Molecular Biology Grade, Promega)

(2) Equipments

- CO<sub>2</sub> incubator (SANYO, MCO-175M)
- Biosafety cabinet (DALTON, NSC-II-A-1200)
- Water bath (Yamato, Thermo-Mate BF400)
- Refrigerated centrifuge (Kubota, 3740)
- Ultrapure water system (Komatsu Electronics, KE0147UC)
- Magnetic stirrer (ADVANTEC, SR500)
- Refrigerator (SANYO, MPR-514 and Panasonic, MPR-162DCN-PJ)
- Freezer (SANYO, MDF-U537D and SANYO, MDF-U332 and SANYO, MDF-U333)
- Deep freezer (Panasonic, MDF-U500VXS5-PJ)
- Automated RNA purification system (QIAGEN, QIAcube)
- Liquid nitrogen tank (CRYOGENIC EQUIPMENT, SR-31)
- NanoDrop One (Thermo Fisher Scientific, ND-ONE-W)
- Micro refrigerated centrifuge (TOMY, MX-205)
- TapeStation (Agilent, 4200 TapeStation)
- Thermal cycler (ProFlex, Thermo Fisher Scientific)
- Dry bath incubator (BSR-MK10, Bio medical science)

(3) Softwares

Excel 2013 and Excel 2019 (Microsoft)

6.4 Culture medium

- Primary culture medium (SkGM™-2)

(1) Composition

[REDACTED]

(2) Preparation

[REDACTED]

- [REDACTED]

(1) Composition

[REDACTED]

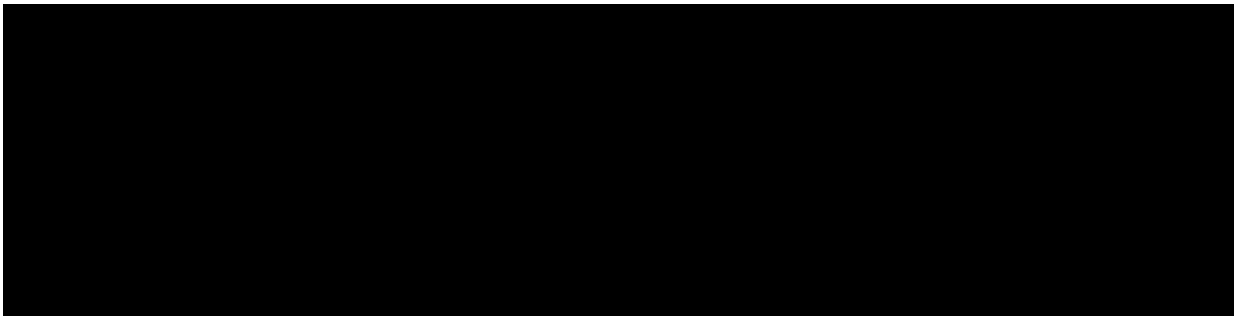
(2) Preparation

[REDACTED]

[REDACTED]

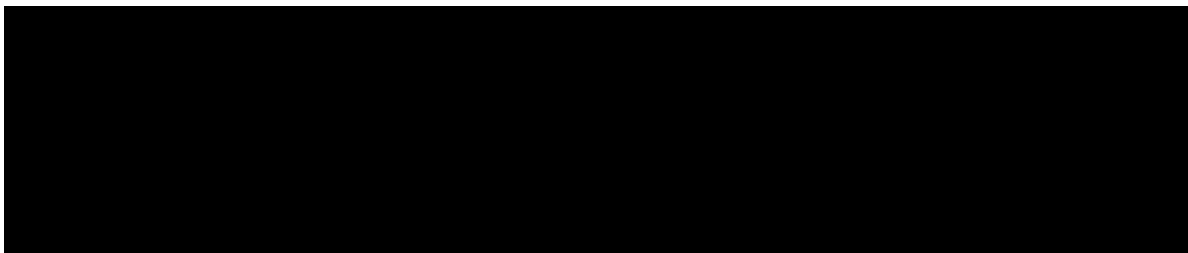
6.5 Cell culture and differentiation

[REDACTED]



6.6 Transfection

6.6.1 Preparation of test substance solution



6.6.2 Preparation of medium containing test substance

6.6.2.1 1<sup>st</sup> run

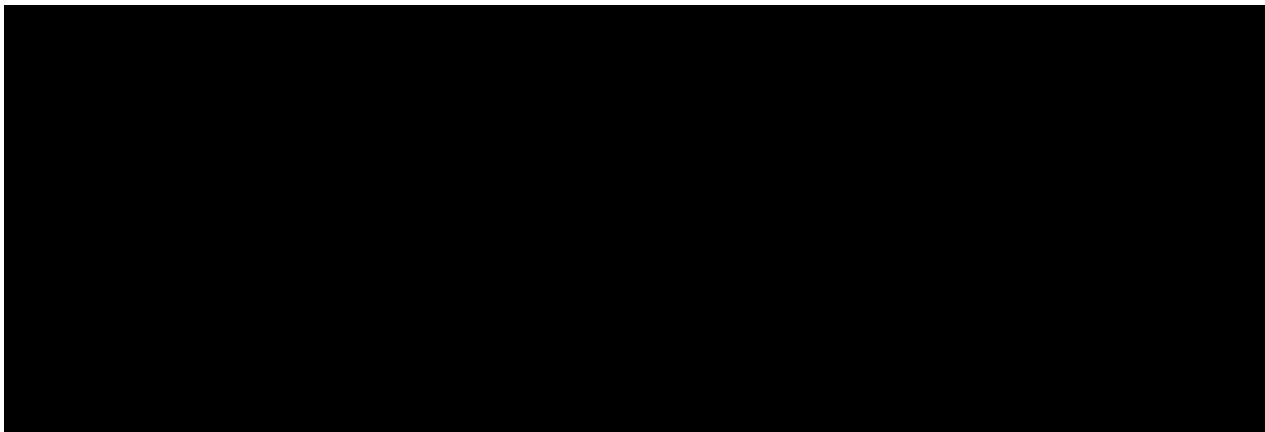


Table 2 Addition volume to prepare Opti-MEM solution for ASO-1.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 µmol/L of ASO-1	13.7 µL <sup>*1</sup>	6.8 µL <sup>*1</sup>
Opti-MEM	486.3 µL	493.2 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 3 Addition volume to prepare Opti-MEM solution for ASO-3.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 $\mu$ mol/L of ASO-3	14.1 $\mu$ L <sup>*1</sup>	7.0 $\mu$ L <sup>*1</sup>
Opti-MEM	485.9 $\mu$ L	493.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 4 Addition volume to prepare Opti-MEM solution for ASO-4.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 $\mu$ mol/L of ASO-4	14.2 $\mu$ L <sup>*1</sup>	7.1 $\mu$ L <sup>*1</sup>
Opti-MEM	485.8 $\mu$ L	492.9 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 5 Addition volume to prepare Opti-MEM solution for ASO-5.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 $\mu$ mol/L of ASO-5	14.5 $\mu$ L <sup>*1</sup>	7.2 $\mu$ L <sup>*1</sup>
Opti-MEM	485.5 $\mu$ L	492.8 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 6 Addition volume to prepare Opti-MEM solution for a mixture of ASO-3, ASO-4 and ASO-5.

	600 nmol/L each as final concentration	300 nmol/L each as final concentration
100 $\mu$ mol/L of ASO-3	14.1 $\mu$ L <sup>*1</sup>	7.0 $\mu$ L <sup>*1</sup>
100 $\mu$ mol/L of ASO-4	14.2 $\mu$ L <sup>*1</sup>	7.1 $\mu$ L <sup>*1</sup>
100 $\mu$ mol/L of ASO-5	14.5 $\mu$ L <sup>*1</sup>	7.2 $\mu$ L <sup>*1</sup>
Opti-MEM	457.2 $\mu$ L	478.6 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

6.6.2.2 2<sup>nd</sup> run

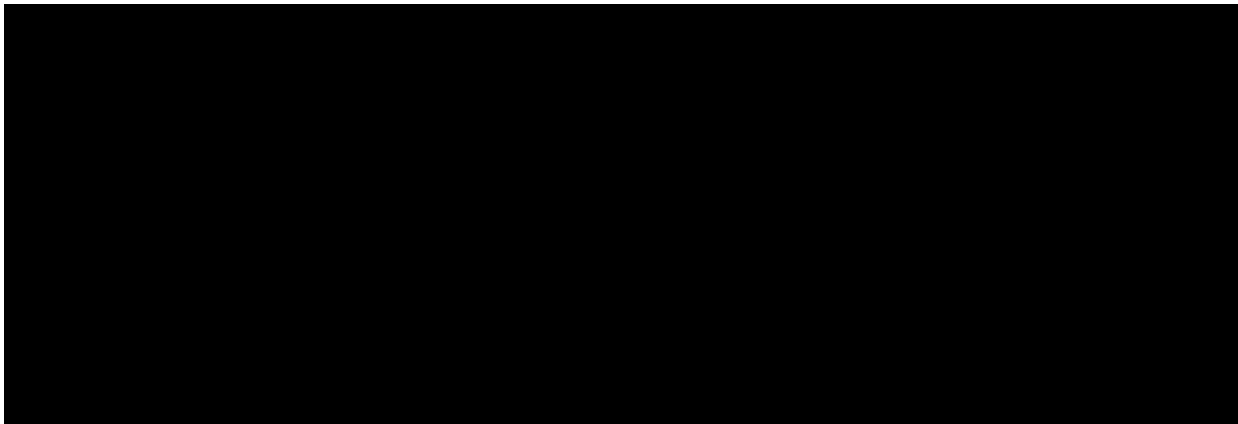


Table 7 Addition volume to prepare Opti-MEM solution for ASO-1.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 µmol/L of ASO-1	14.2 µL <sup>*1</sup>	7.1 µL <sup>*1</sup>
Opti-MEM	485.8 µL	492.9 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 8 Addition volume to prepare Opti-MEM solution for ASO-3.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 µmol/L of ASO-3	14.1 µL <sup>*1</sup>	7.1 µL <sup>*1</sup>
Opti-MEM	485.9 µL	492.9 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 9 Addition volume to prepare Opti-MEM solution for ASO-4.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 µmol/L of ASO-4	14.3 µL <sup>*1</sup>	7.1 µL <sup>*1</sup>
Opti-MEM	485.7 µL	492.9 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 10 Addition volume to prepare Opti-MEM solution for ASO-5.

	600 nmol/L as final concentration	300 nmol/L as final concentration
100 µmol/L of ASO-5	13.3 µL <sup>*1</sup>	6.6 µL <sup>*1</sup>
Opti-MEM	486.7 µL	493.4 µL

<sup>\*1</sup>: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 11 Addition volume to prepare Opti-MEM solution for a mixture of ASO-3, ASO-4 and ASO-5.

	600 nmol/L each as final concentration	300 nmol/L each as final concentration
100 µmol/L of ASO-3	14.1 µL <sup>*1</sup>	7.1 µL <sup>*1</sup>
100 µmol/L of ASO-4	14.3 µL <sup>*1</sup>	7.1 µL <sup>*1</sup>
100 µmol/L of ASO-5	13.3 µL <sup>*1</sup>	6.6 µL <sup>*1</sup>
Opti-MEM	458.3 µL	479.2 µL

<sup>\*1</sup>: This volume was calculated by the actual concentration measured by NanoDrop One.

6.6.3 Preparation of Lipofectin in Opti-MEM

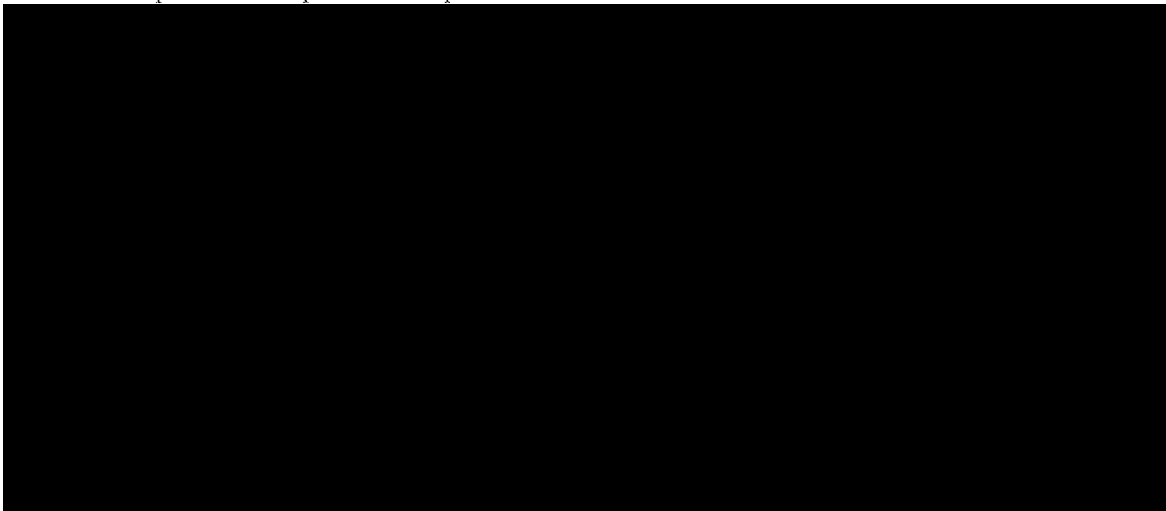
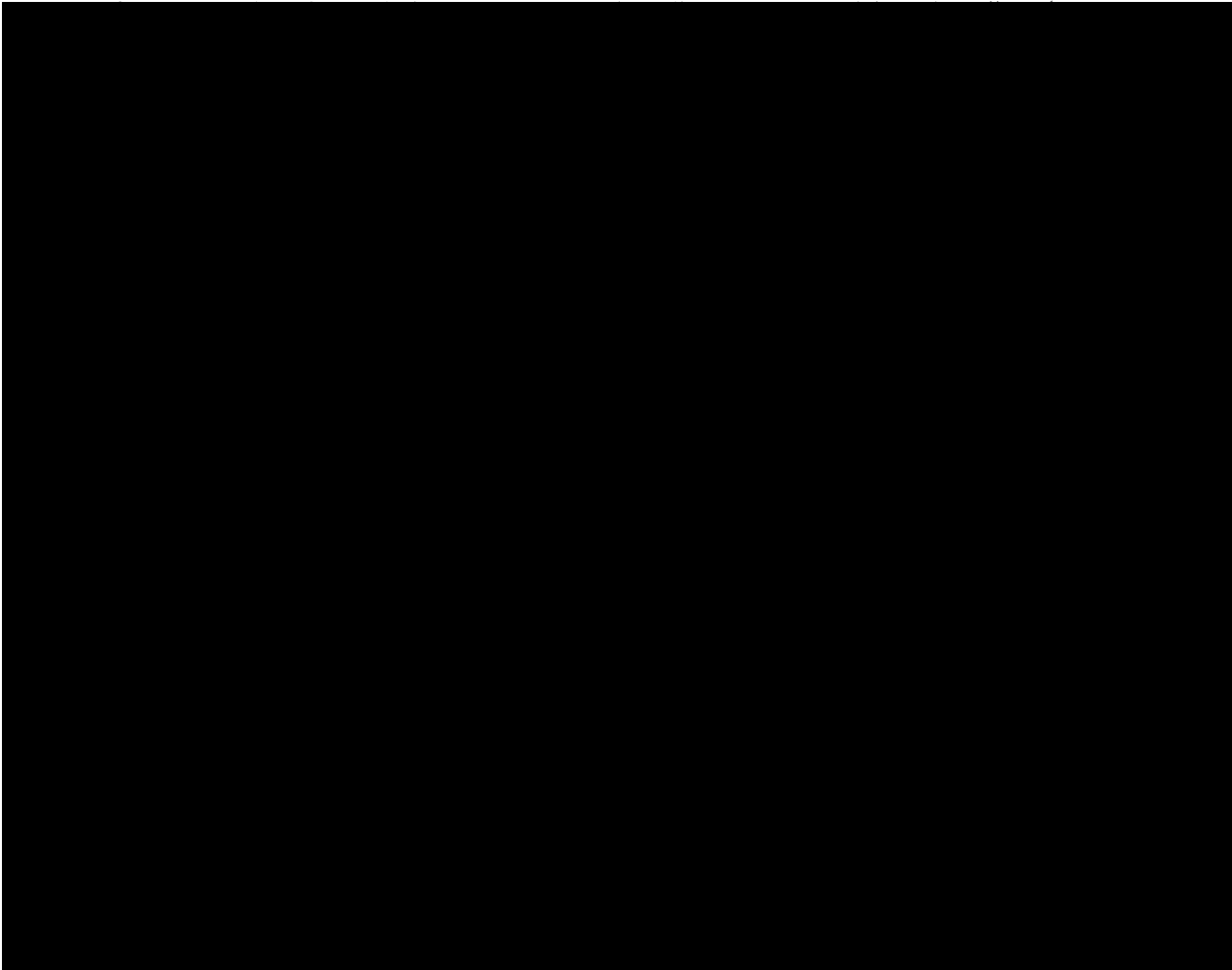


Table 12 Addition volume to prepare Lipofectin in Opti-MEM

Final concentration of ASO	0 nmol/L	600 nmol/L or mixture of 600 nmol/L of ASO-3, ASO-4 and ASO-5	300 nmol/L or mixture of 300 nmol/L of ASO-3, ASO-4 and ASO-5	mixture of 600 nmol/L of ASO-3, ASO-4 and ASO-5	mixture of 300 nmol/L of ASO-3, ASO-4 and ASO-5
Final concentration of Lipofectin	0 µL/mL	12 µL/mL	6 µL/mL	36 µL/mL	18 µL/mL
Lipofectin	0 µL	30 µL	15 µL	90 µL	45 µL
Opti-MEM	500 µL	470 µL	485 µL	410 µL	455 µL
Concentration of Lipofectin in Opti-MEM	0 µL/mL	60 µL/mL	30 µL/mL	180 µL/mL	90 µL/mL

6.6.4 Exposure





	1	2	3	4	5	6
A	0 nmol/L			600 nmol/L of ASO-1 and 12 µL/mL of Lipofectin		
B	600 nmol/L of ASO-3 and 12 µL/mL of Lipofectin			600 nmol/L of ASO-4 and 12 µL/mL of Lipofectin		
C	600 nmol/L of ASO-5 and 12 µL/mL of Lipofectin			mixture of ASO-3, 4, 5 at 600 nmol/L each and 12 µL/mL of Lipofectin		
D	mixture of ASO-3, 4, 5 at 600 nmol/L each and 36 µL/mL of Lipofectin			_*2		

Fig. 1 Plate layout\*1 of exposure to ASOs at 600 nmol/L

-: filled with medium

\*1: Concentrations provided in the plate layout were as final concentration in each well.

\*2: There was no cells in wells and only 500 µL of fresh Opti-MEM without Lipofectin was added at the time of transfection

	1	2	3	4	5	6
A	0 nmol/L			300 nmol/L of ASO-1 and 6 µL/mL of Lipofectin		
B	300 nmol/L of ASO-3 and 6 µL/mL of Lipofectin			300 nmol/L of ASO-4 and 6 µL/mL of Lipofectin		
C	300 nmol/L of ASO-5 and 6 µL/mL of Lipofectin			mixture of ASO-3, 4, 5 at 300 nmol/L each and 6 µL/mL of Lipofectin		
D	mixture of ASO-3, 4, 5 at 300 nmol/L each and 18 µL/mL of Lipofectin			_*2		

Fig. 2 Plate layout\*1 of exposure to ASOs at 300 nmol/L

-: filled with medium

\*1: Concentrations provided in the plate layout were as final concentration in each well.

\*2: There was no cells in wells and only 500 µL of fresh Opti-MEM without Lipofectin was added at the time of transfection

## 6.7 Gene Expression analysis

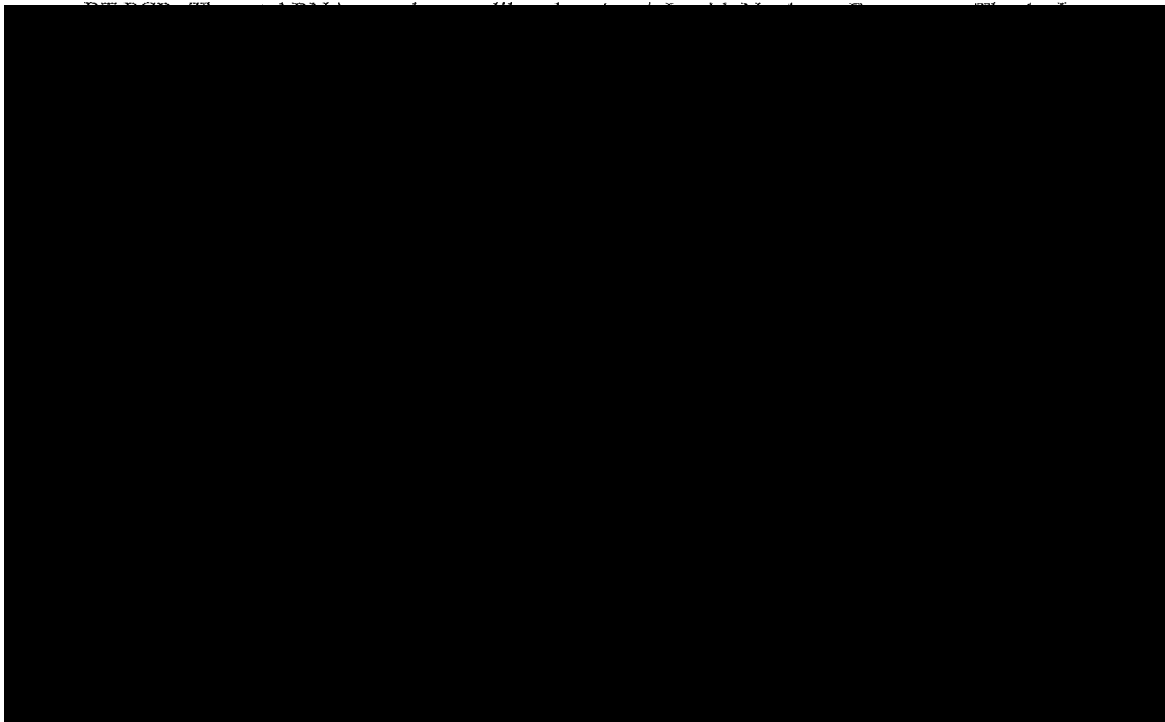
### 6.7.1 RNA isolation and RNA concentration measurement



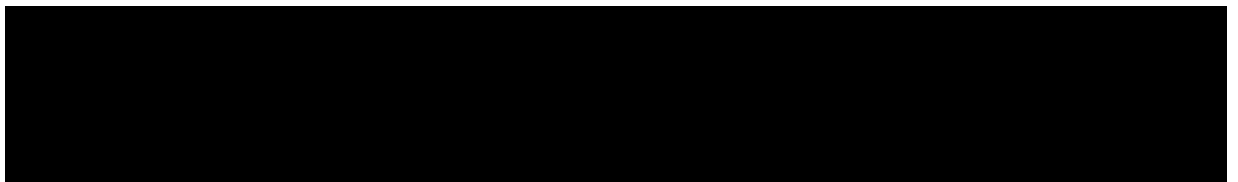
### 6.7.2 RNA quality analysis by the 4200 TapeStation



### 6.7.3 cDNA synthesis



### 6.7.4 Electrophoresis





*D*: Diluted factor

Note: The exon skipping activities of each ASOs were evaluated in two independent exposure samples using the same test substance solutions.

7. RESULT

7.1 Concentration of ASO solutions

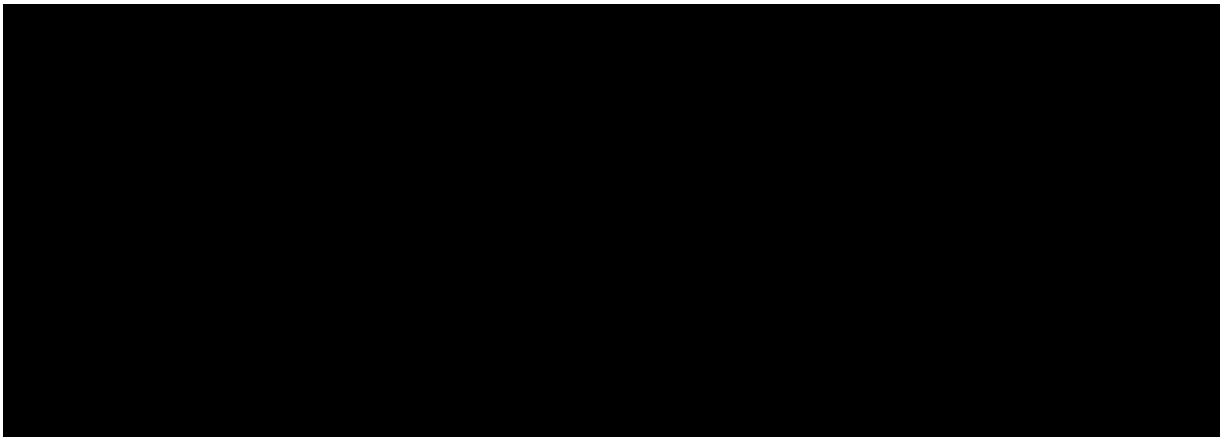
7.1.1 1<sup>st</sup> run



Table 13 Actual concentration of ASO solutions

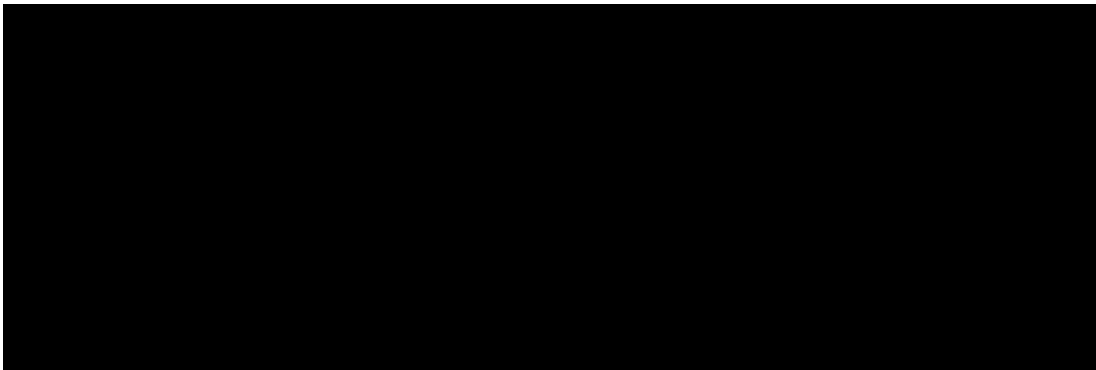
A large black rectangular redaction box covering the content of Table 13.

7.1.2 2<sup>nd</sup> run



7.2 Assessment of skipping efficiency of ASOs

7.2.1 1<sup>st</sup> run



(N/A)" – 1.0% at 300 nmol/L and 5.7 – 8.7% at 600 nmol/L (Fig. 17 – 24 and Table 18). The exon skipping efficiencies were 41.2 – 46.7% for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (6 µL/mL of Lipofectin) and 52.5 – 60.8% for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (12 µL/mL of Lipofectin) (Fig. 25 – 31 and Table 19). The exon skipping efficiencies were 61.8 – 64.1% for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (18 µL/mL of Lipofectin) and 83.1 – 86.8% for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (36 µL/mL of Lipofectin) (Fig. 32 – 36 and Table 20).

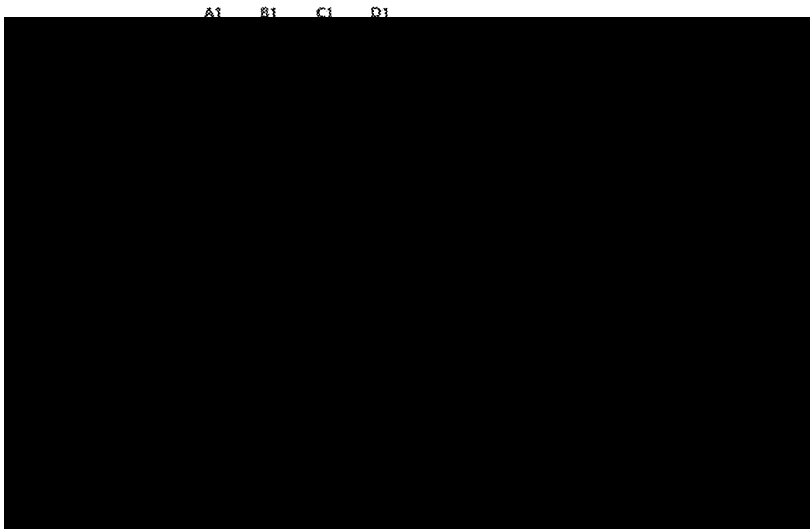
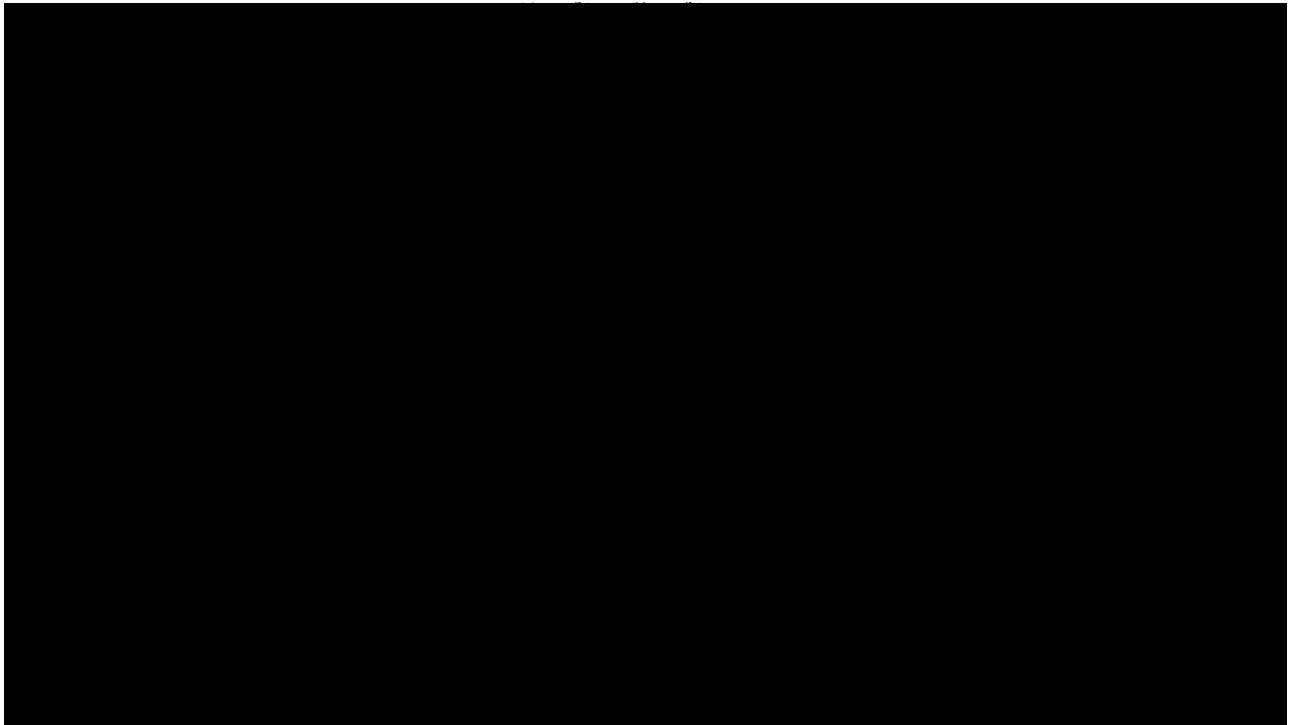
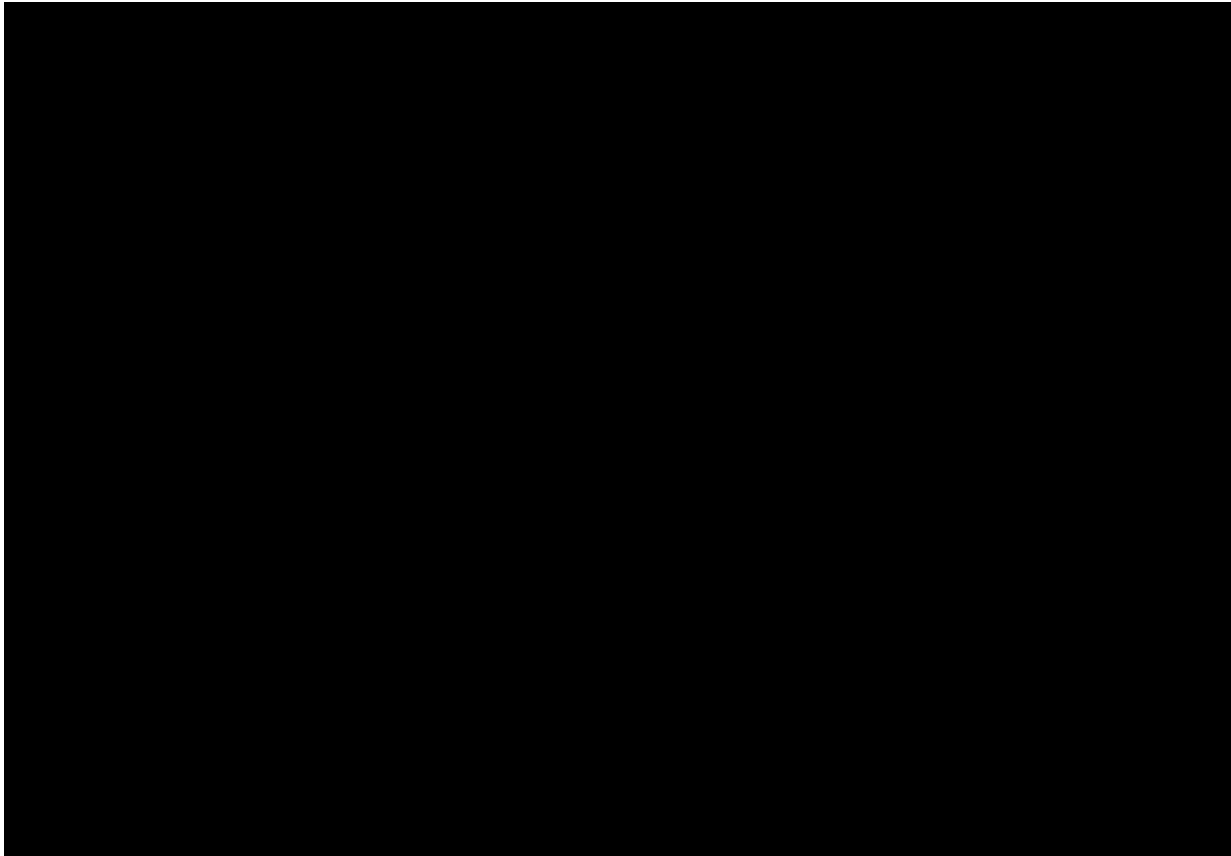
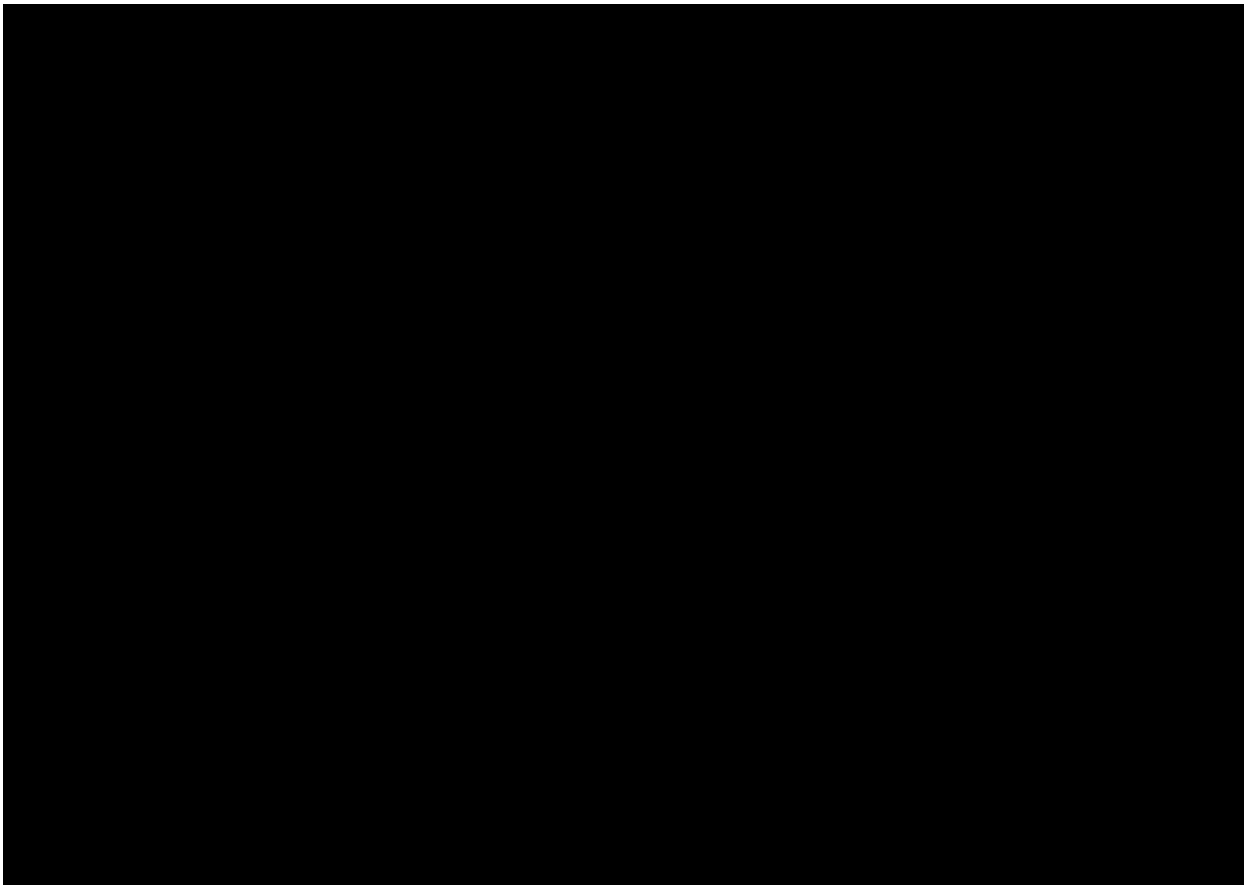
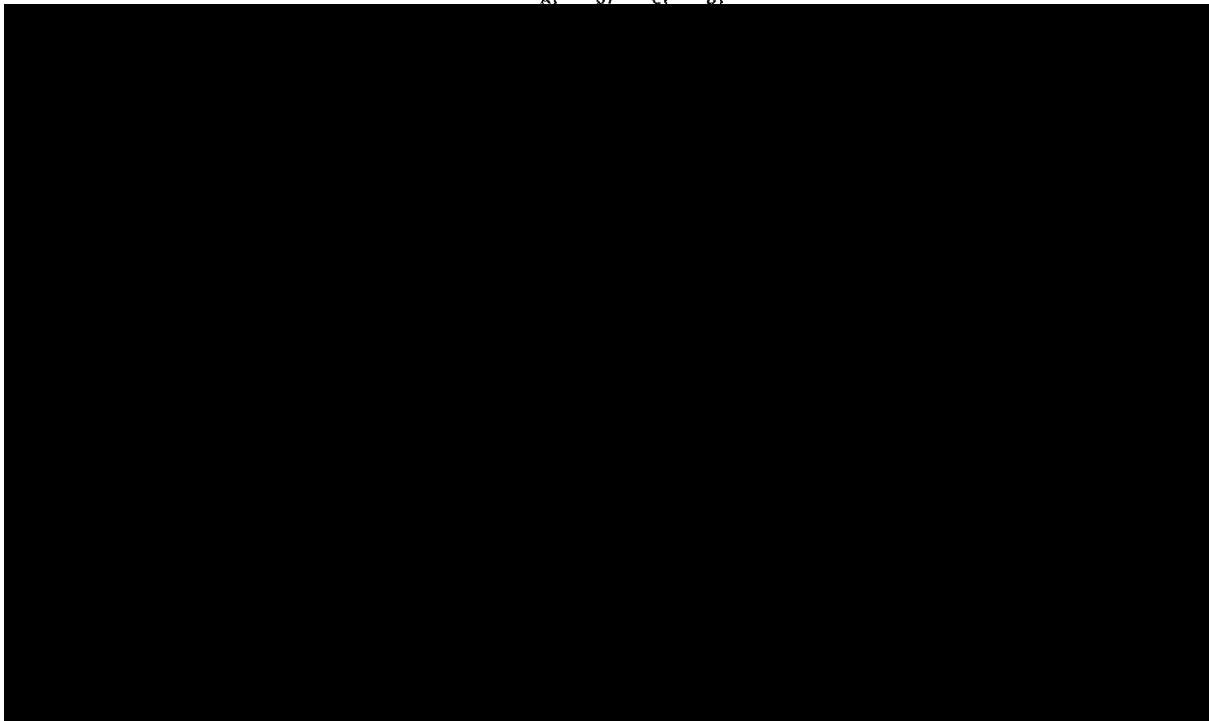


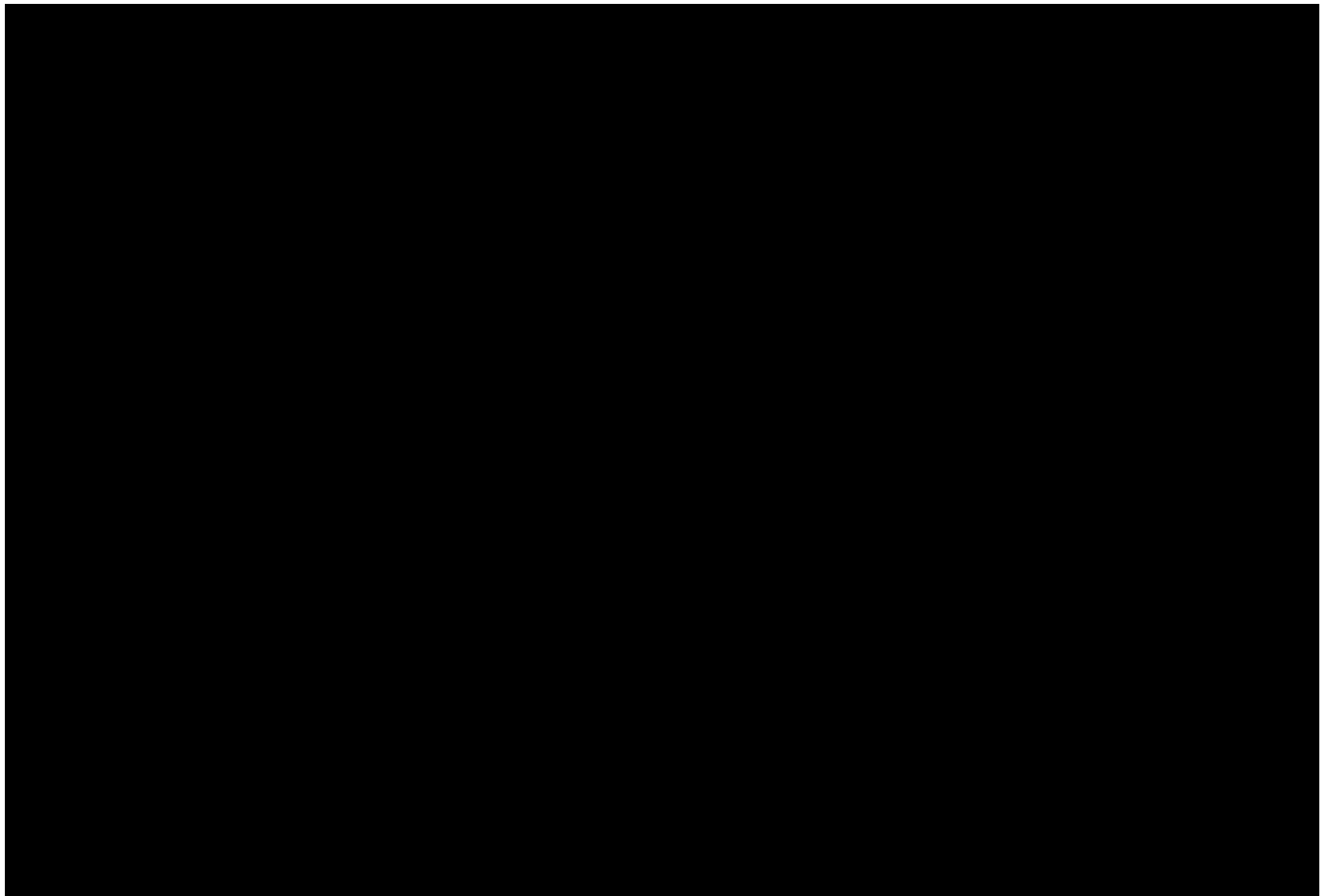
Fig. 3 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-1 exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-1 at 300 nmol/L (replicate 1, 2 and 3, respectively)



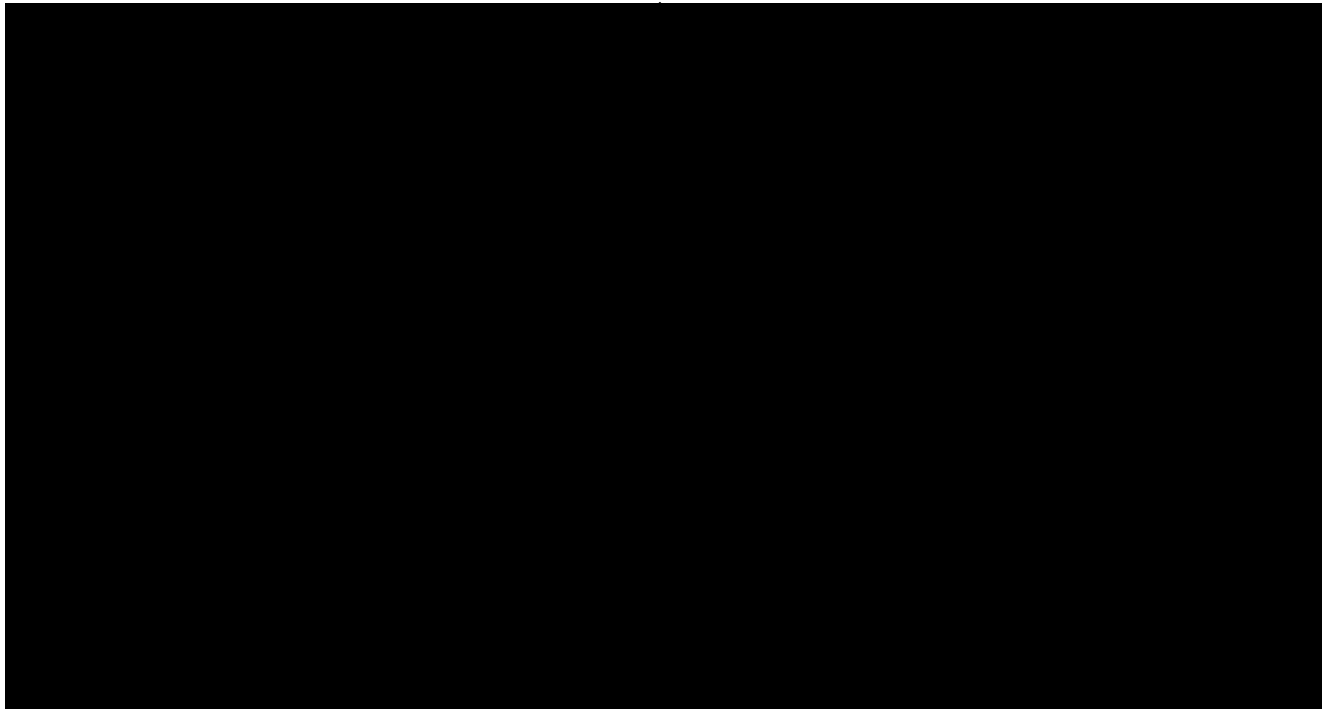


A1 B1 C1 D1





A1 B1





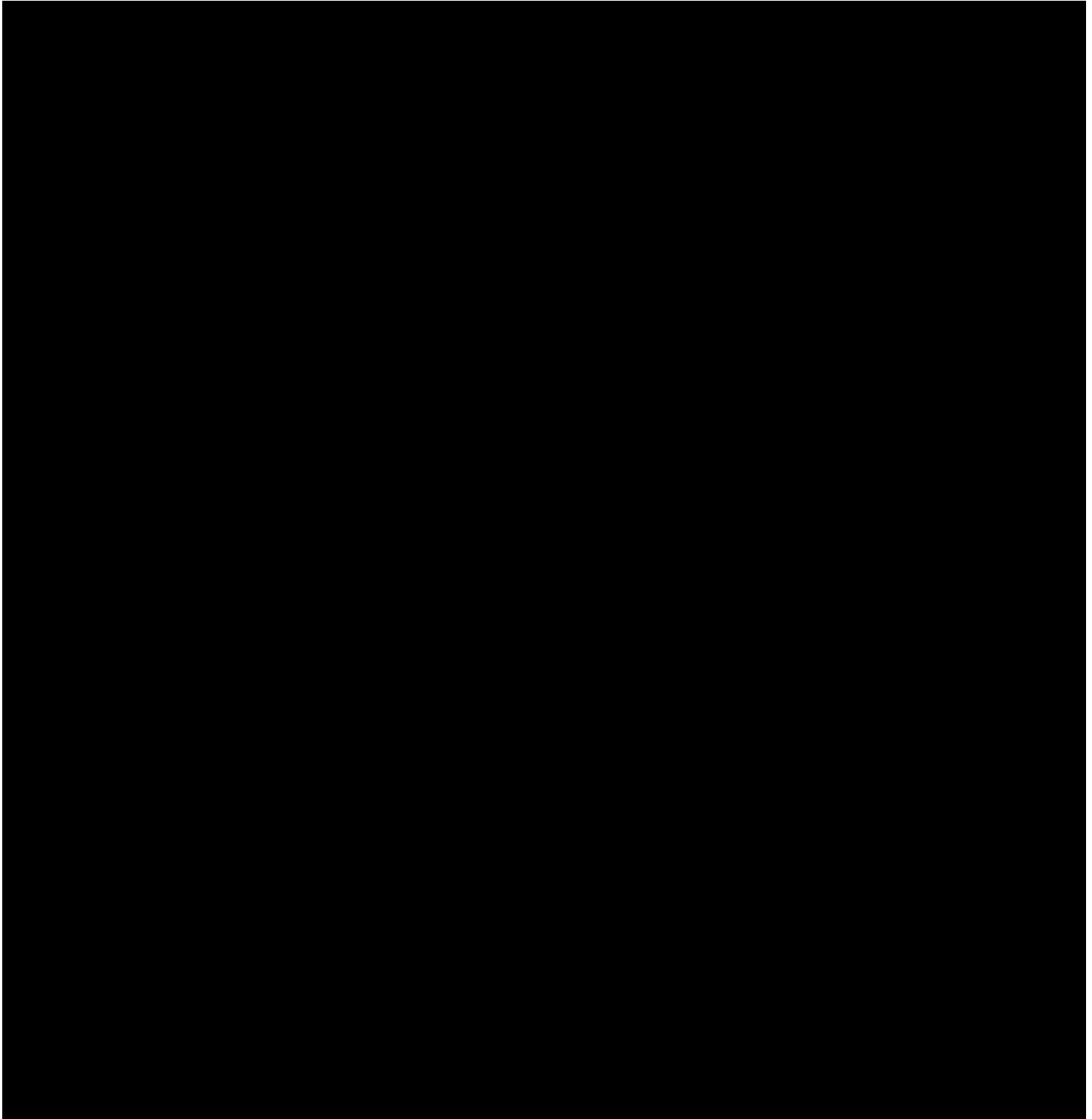


Fig. 11 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-3 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-3 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-3 at 600 nmol/L (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

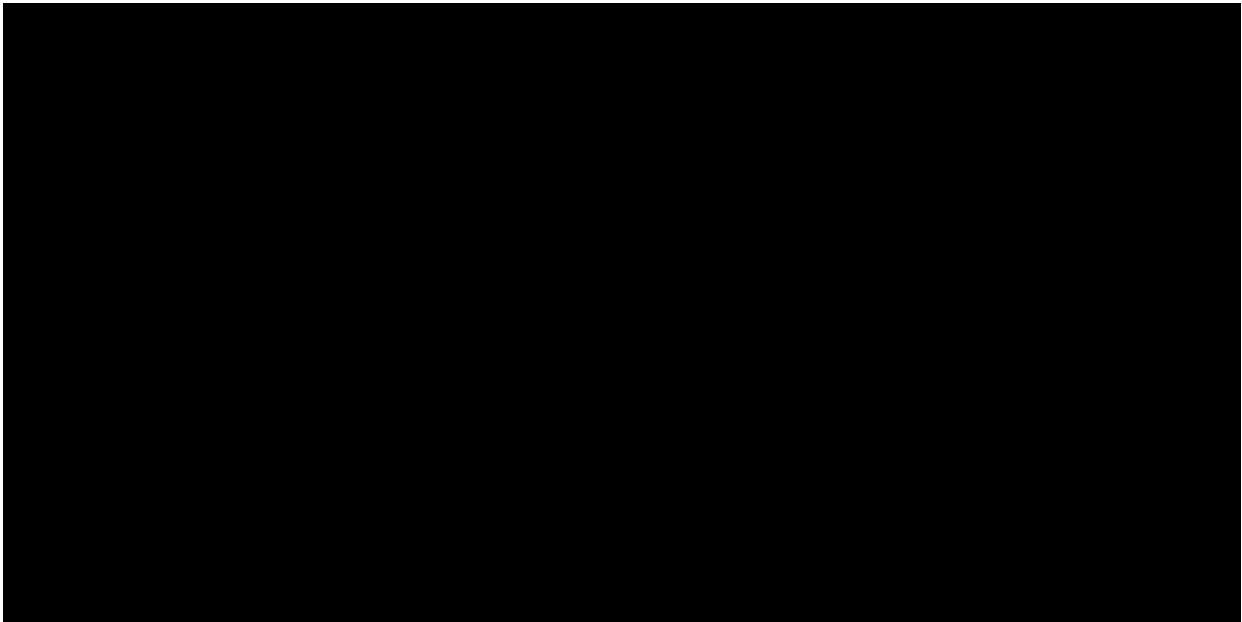


Fig. 12 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively)

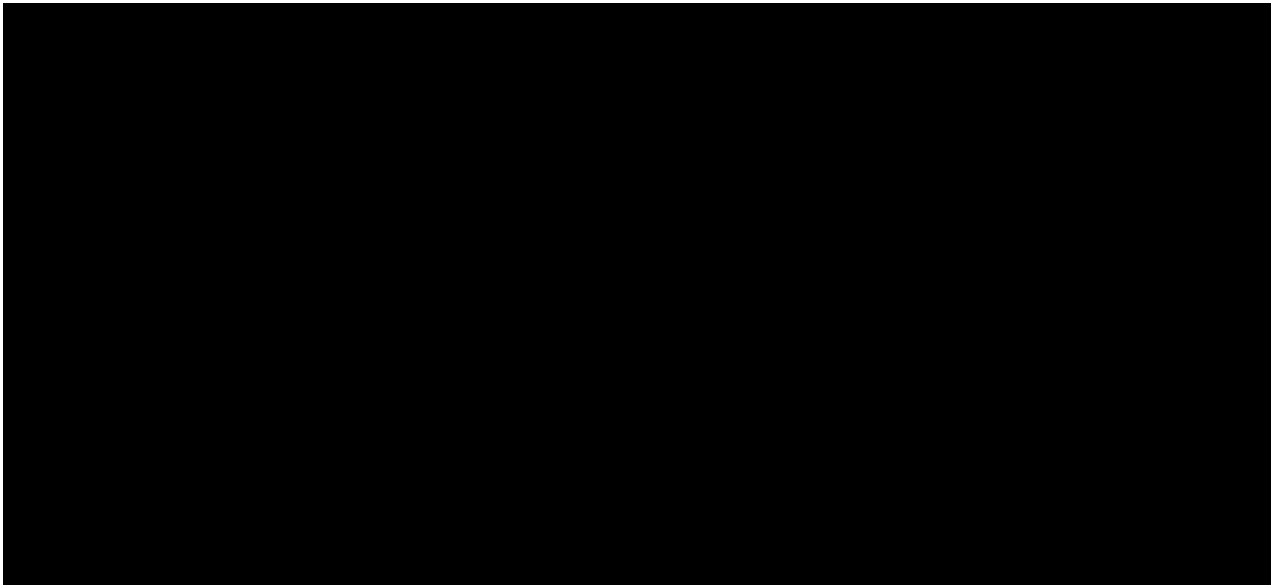


Fig. 13 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively)

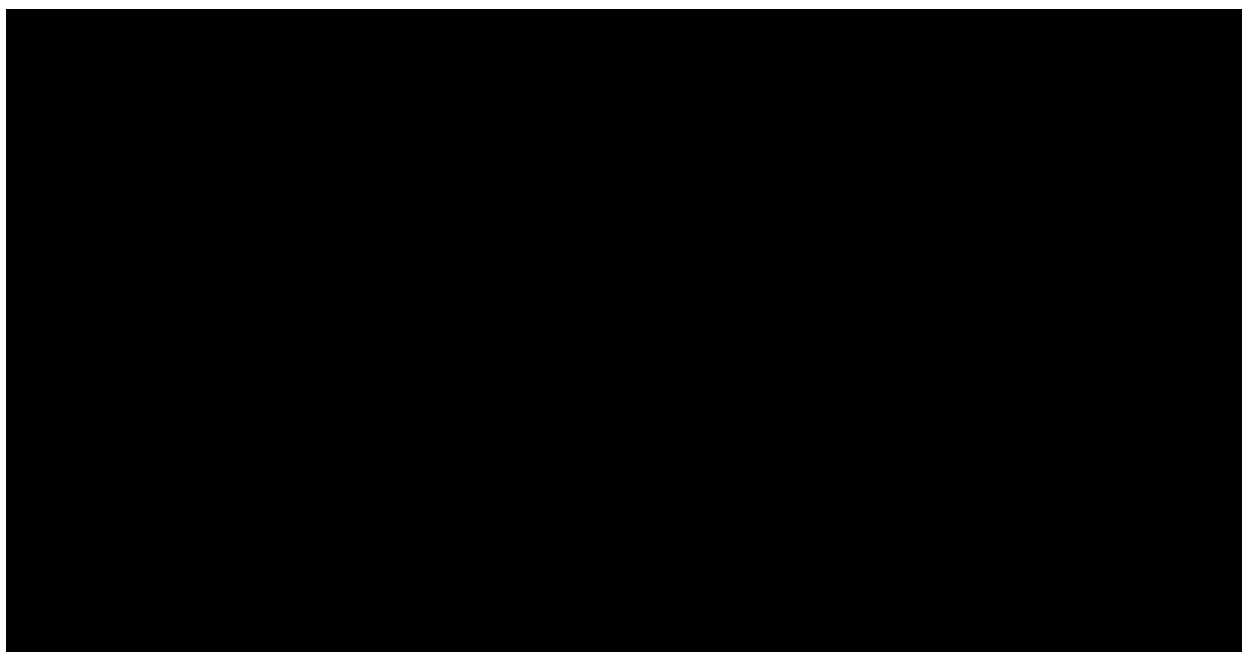


Fig. 14 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: ASO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

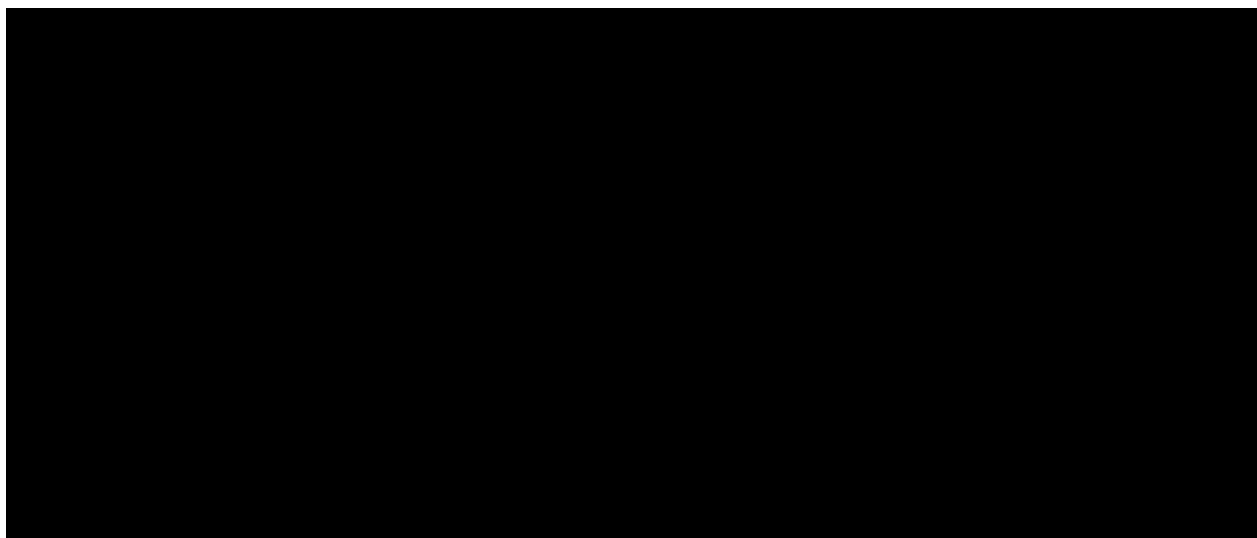


Fig. 15 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 600 nmol/L (replicate 1, 2 and 3, respectively)

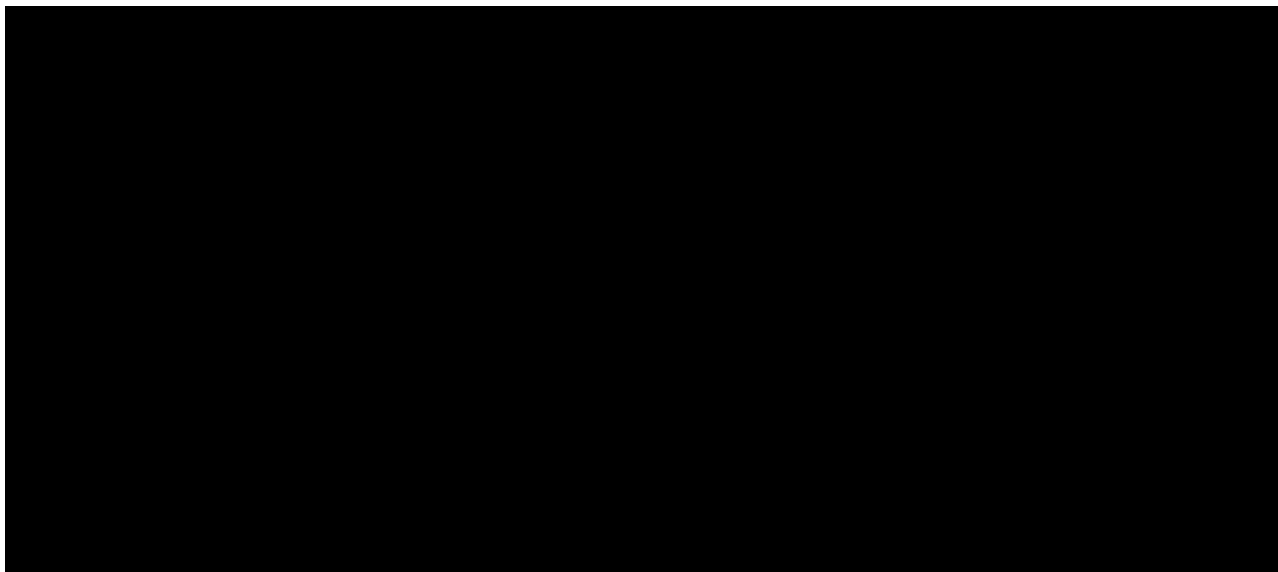


Fig. 16 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-4 at 600 nmol/L (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

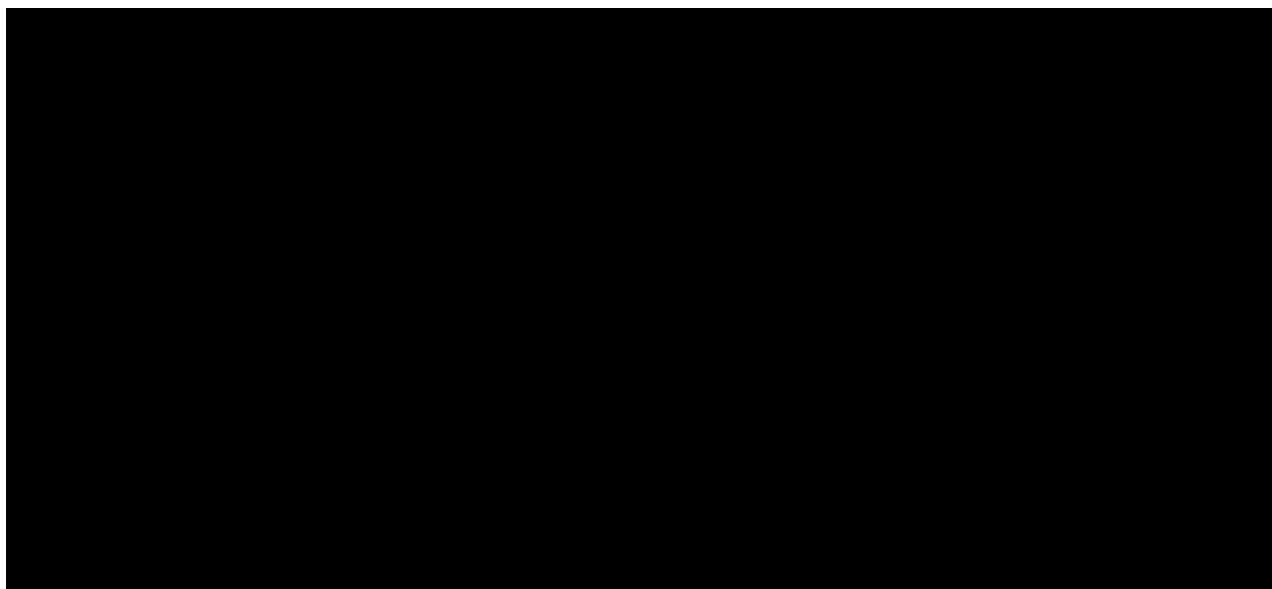


Fig. 17 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

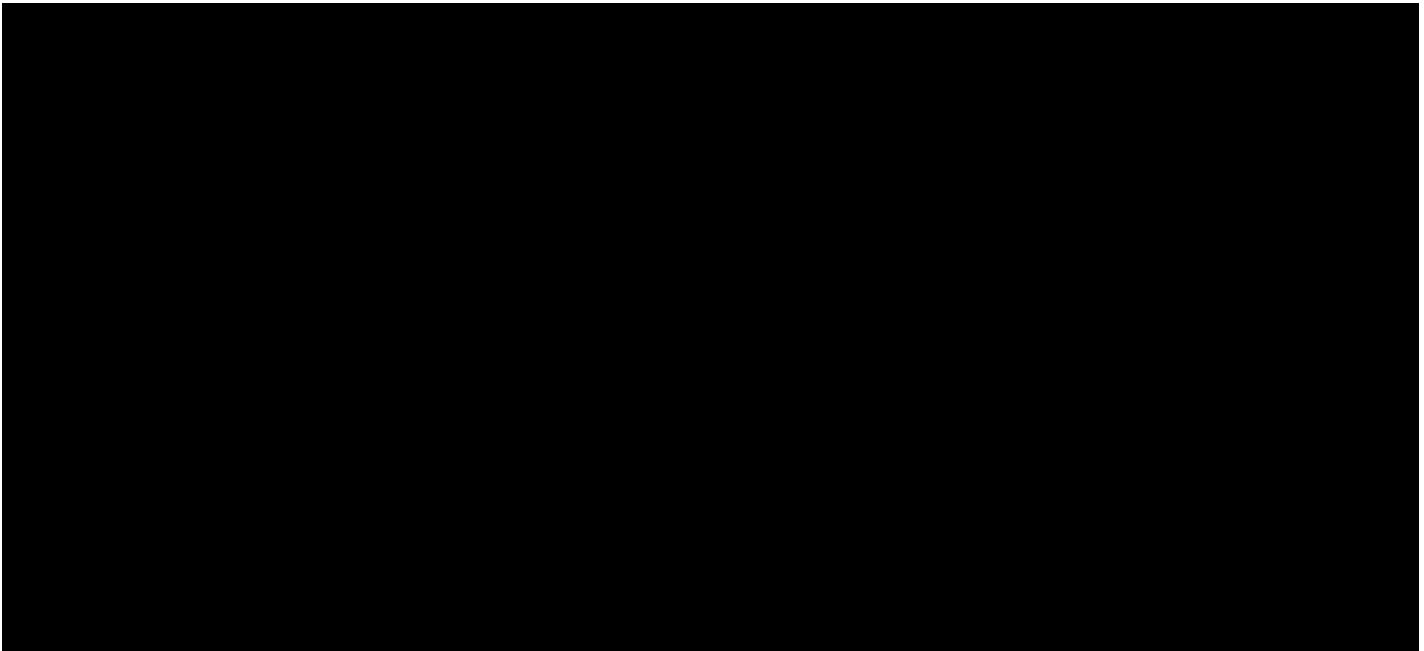


Fig. 18 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

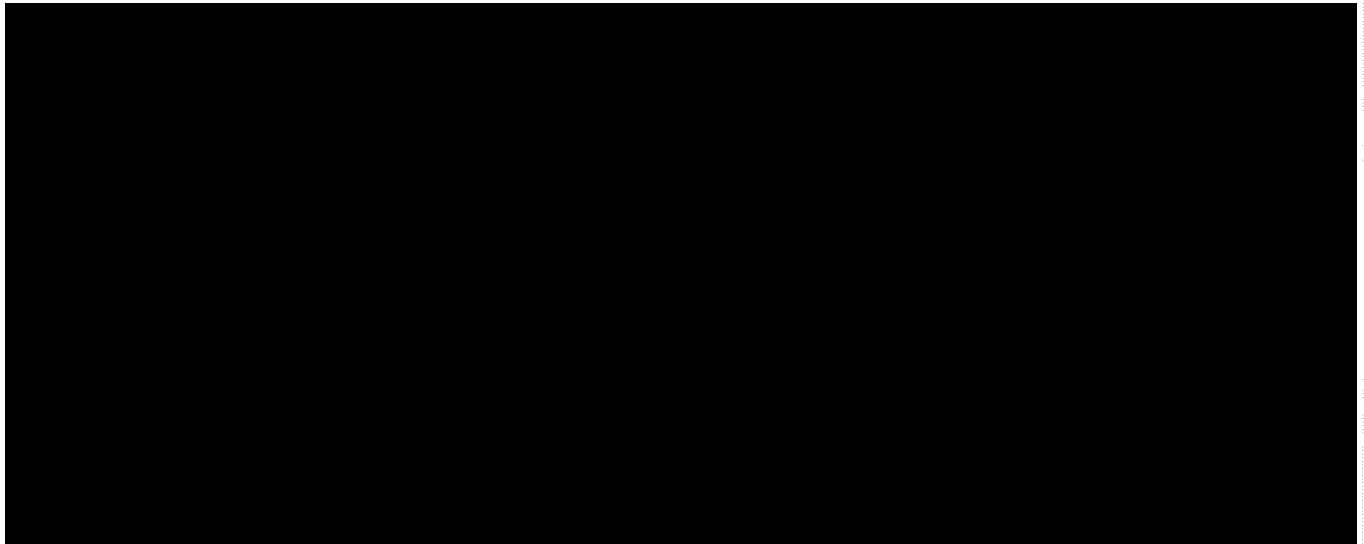


Fig. 19 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1 and C1: ASO-5 at 300 nmol/L (replicate 1 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

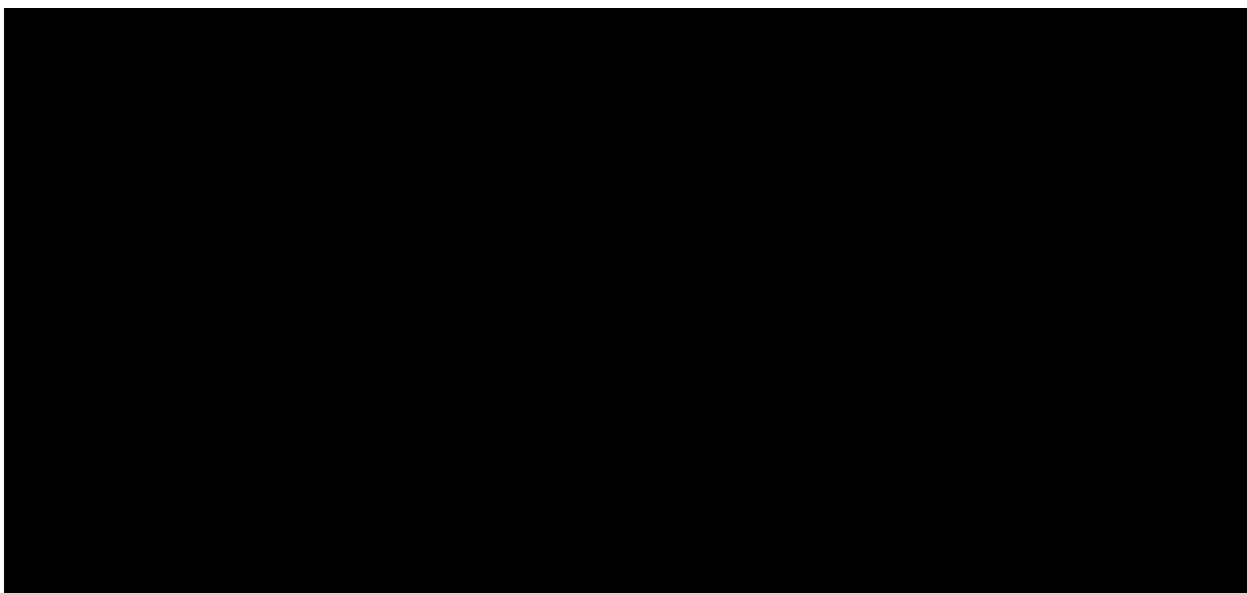


Fig. 20 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1: ASO-5 at 300 nmol/L (replicate 2)

Exclamation mark indicate that a noise peak was detected outside upper or lower marker.

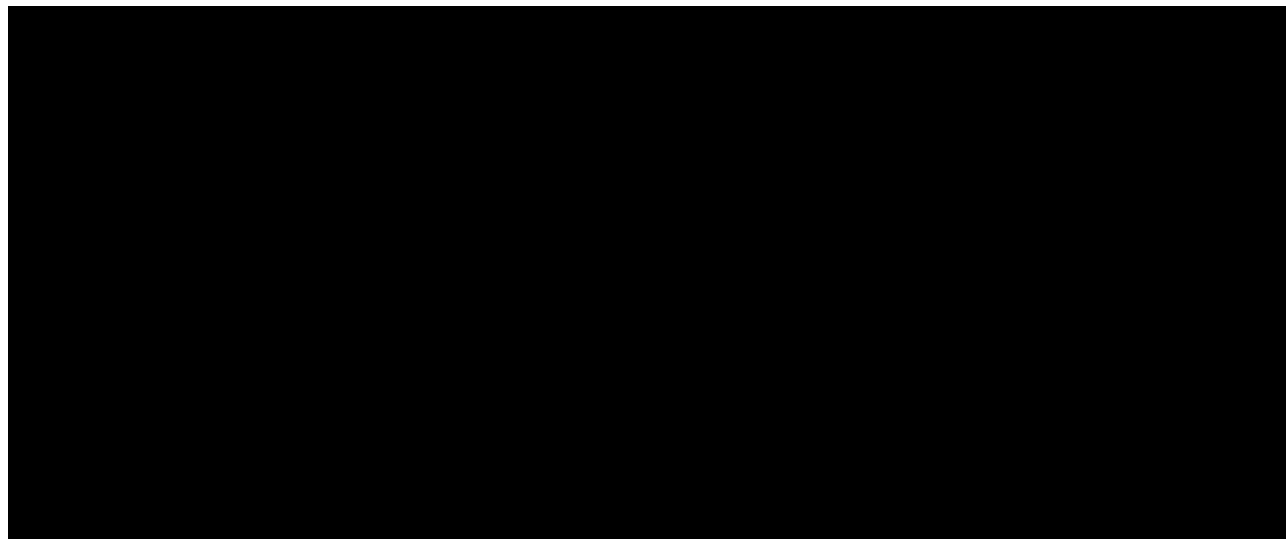


Fig. 21 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1 and C1: ASO-5 at 300 nmol/L (replicate 1 and 3, respectively)

Exclamation mark indicate that a noise peak was detected outside upper or lower marker.

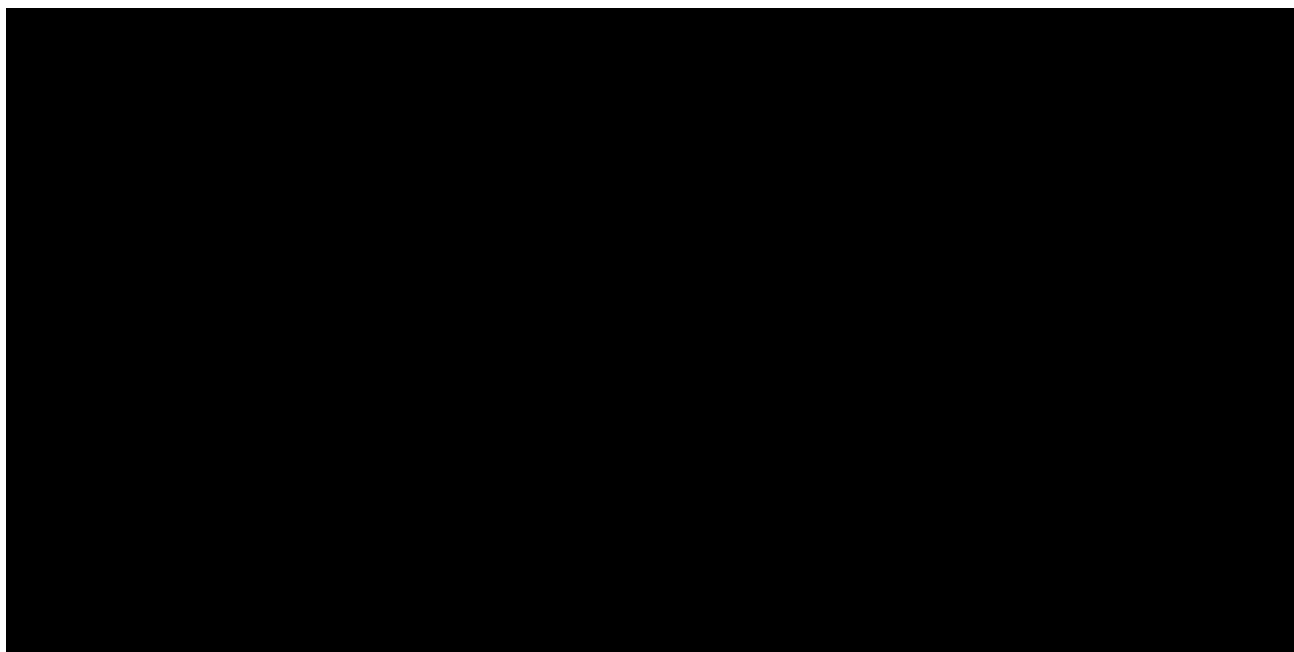


Fig. 22 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)  
A1: Ladder Marker, B1: ASO-5 at 300 nmol/L (replicate 2)

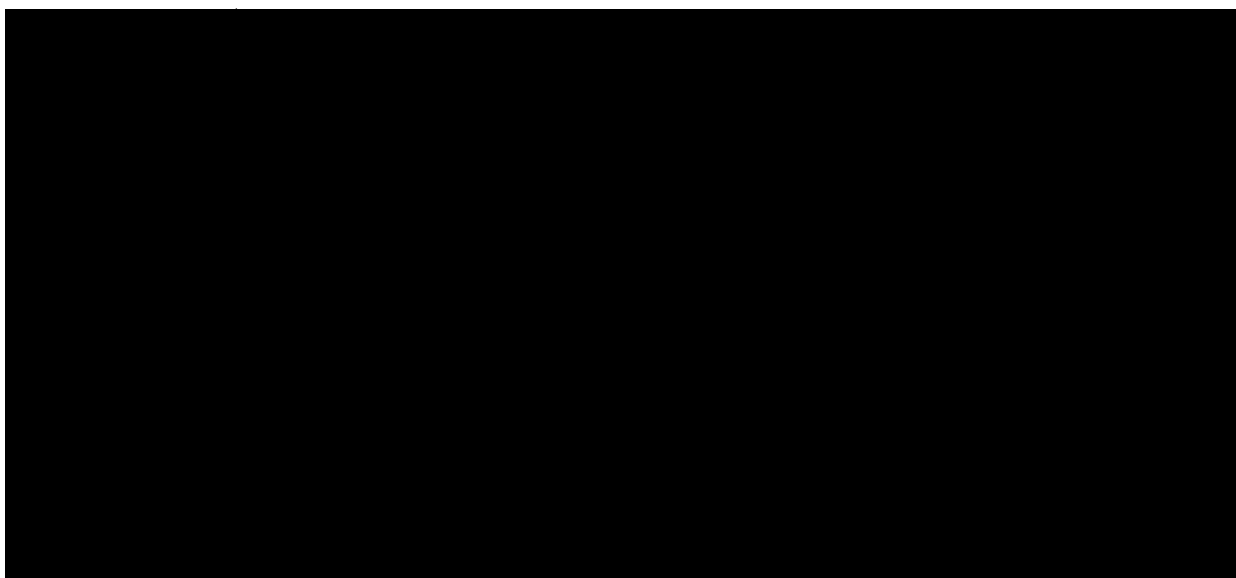


Fig. 23 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

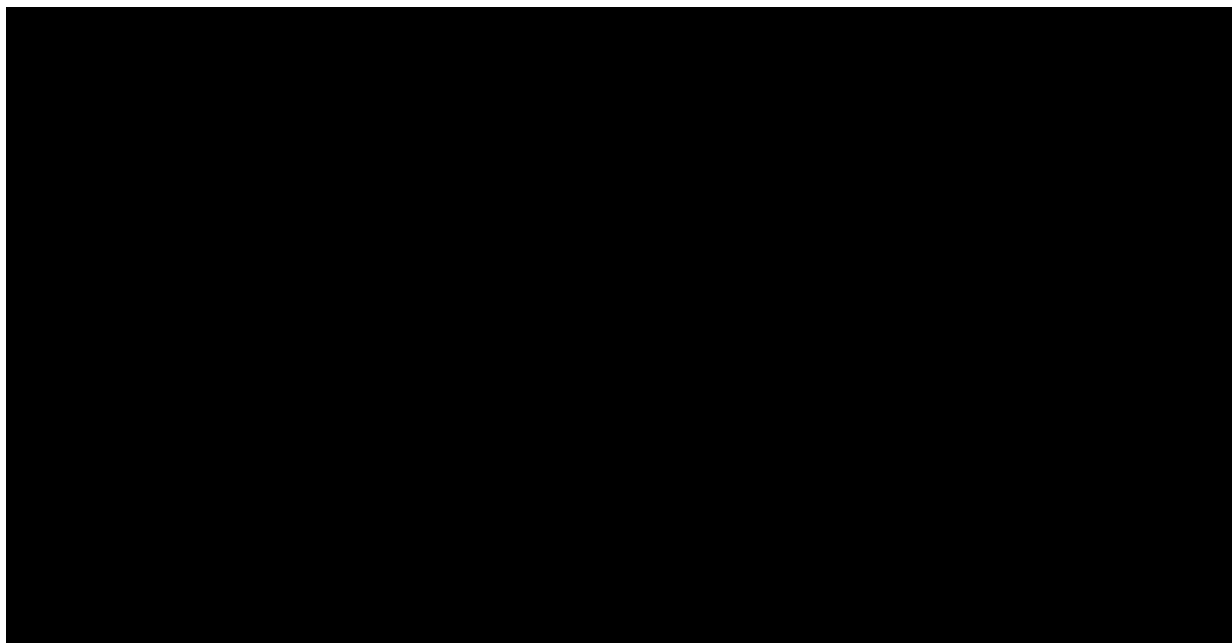


Fig. 24 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

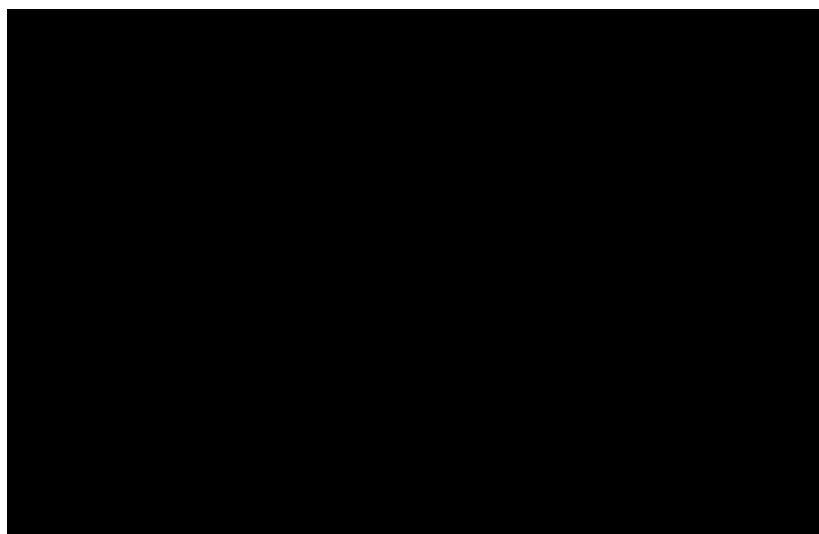


Fig. 25 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (6  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)



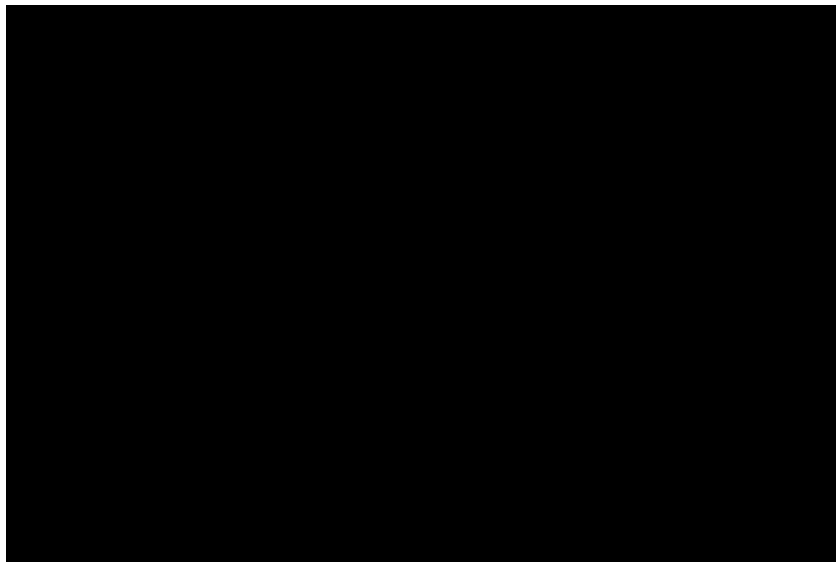


Fig. 26 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (6  $\mu\text{L/mL}$  of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

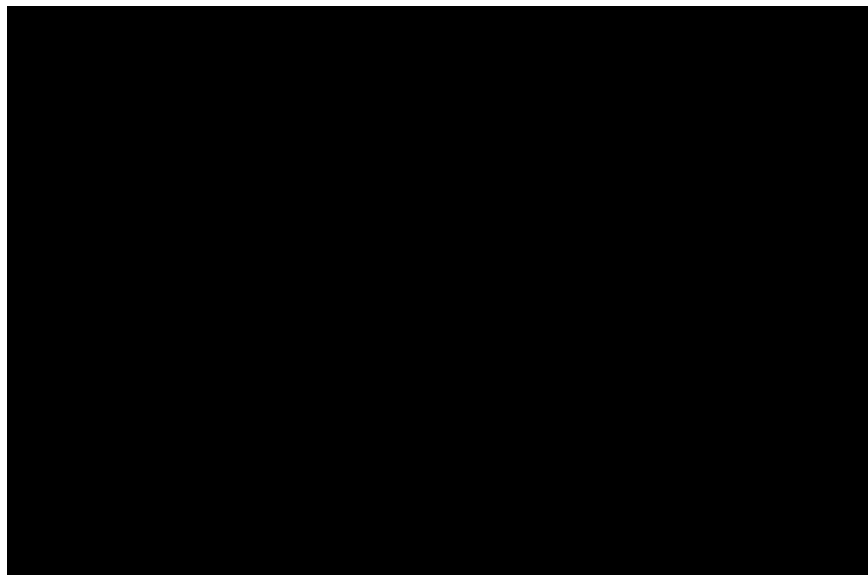


Fig. 27 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (6  $\mu\text{L/mL}$  of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 20, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.



Fig. 28 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (12  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

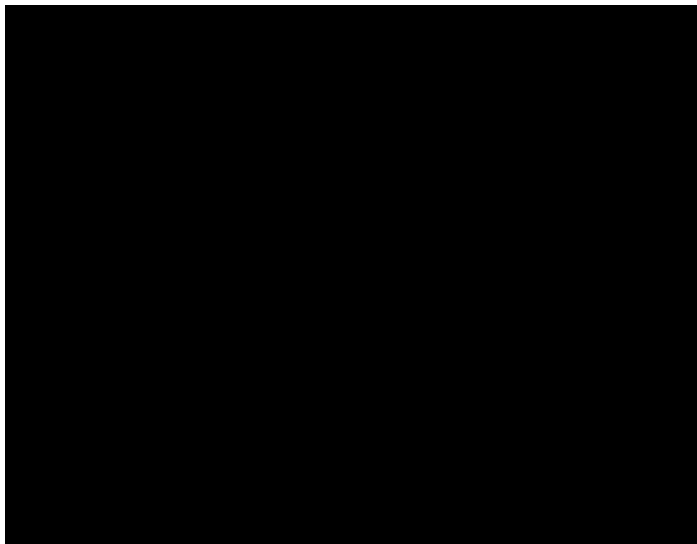


Fig. 29 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (12  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

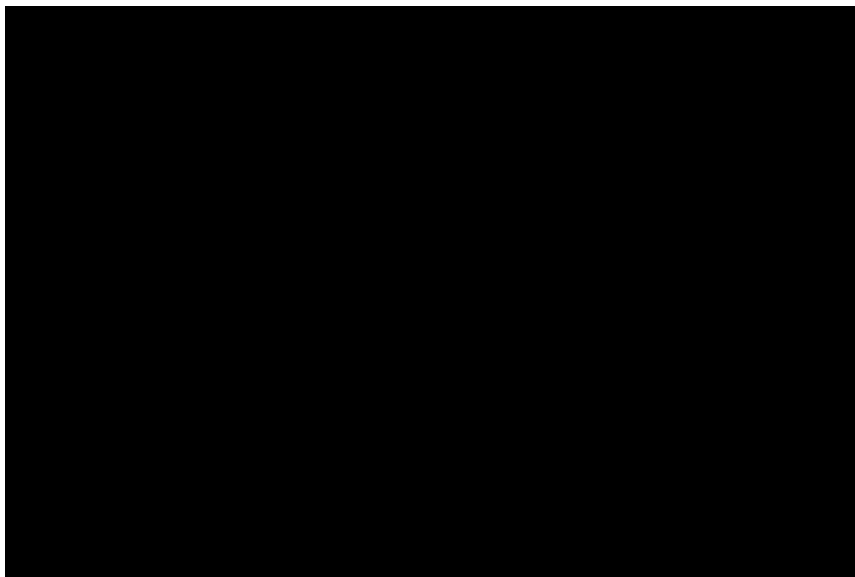


Fig. 30 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (12  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 10, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1: mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1)

Exclamation mark indicate that a noise peak was detected outside upper or lower marker.

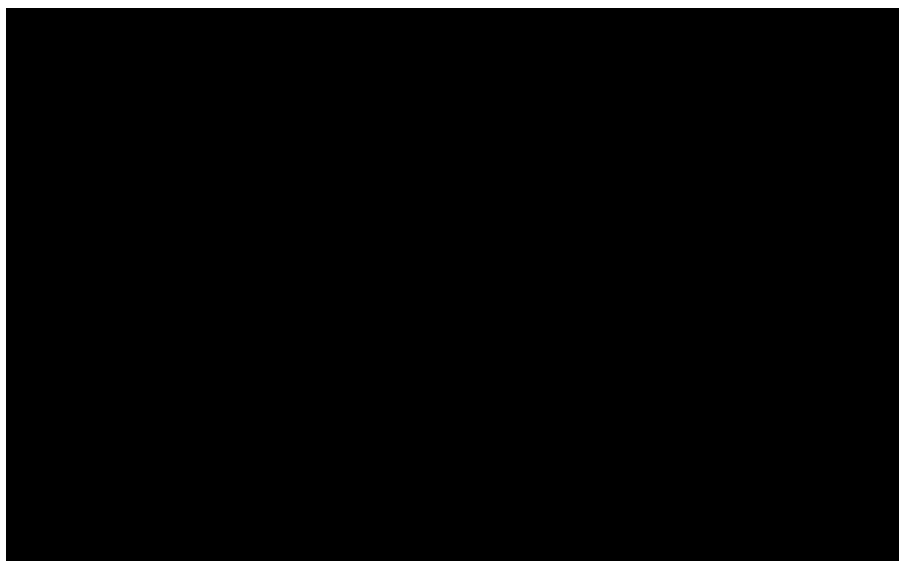


Fig. 31 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (12  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 10, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1 and C1: mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

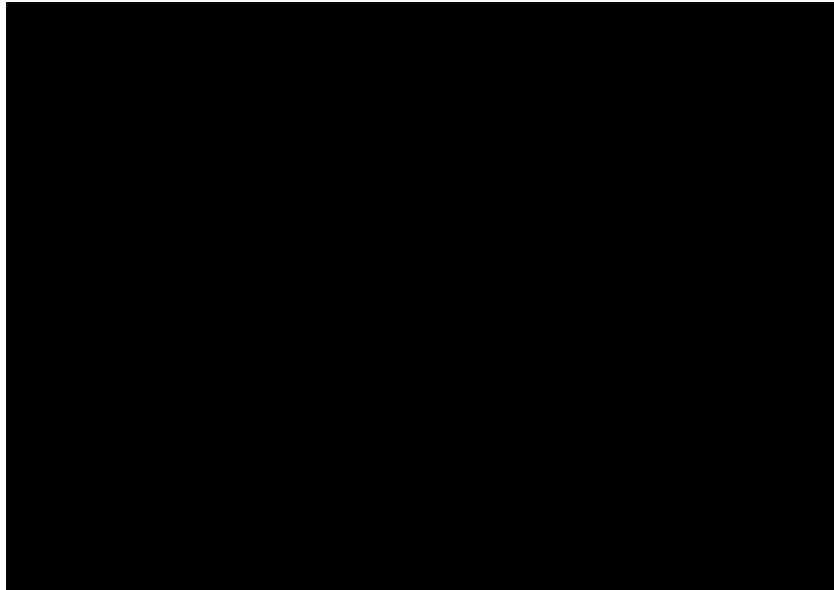


Fig. 32 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (18  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

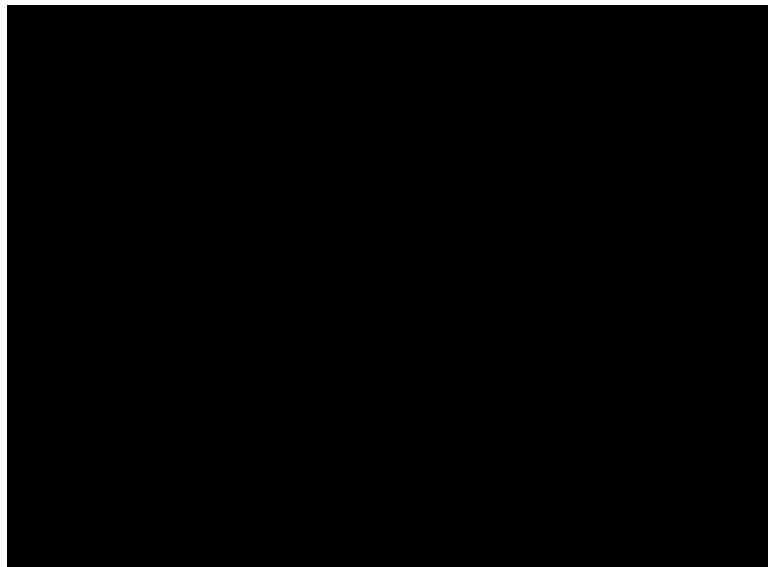


Fig. 33 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (18  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

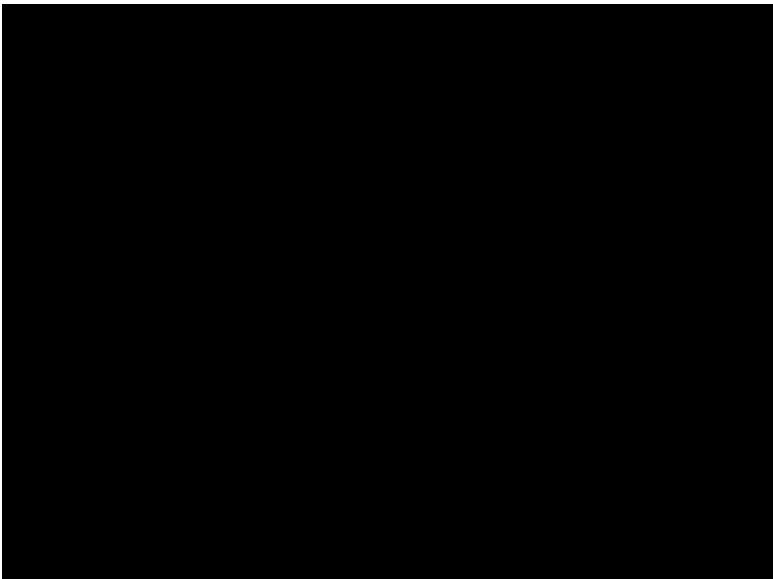


Fig. 34 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (36  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

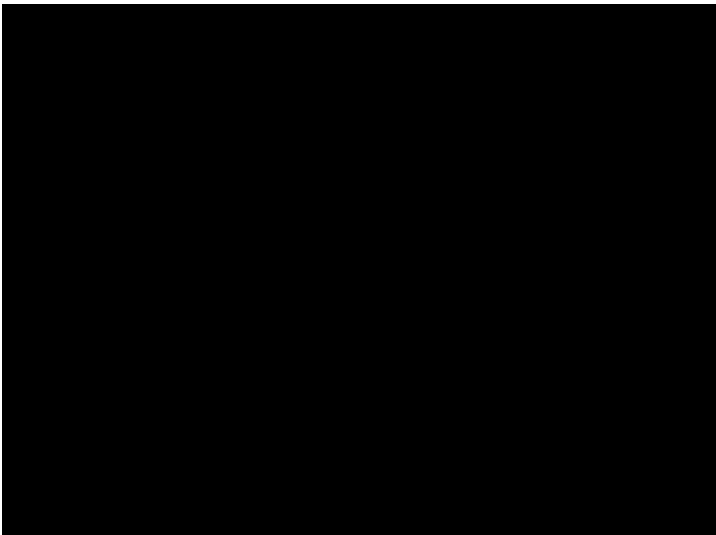


Fig. 35 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (36  $\mu$ L/mL of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 100, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

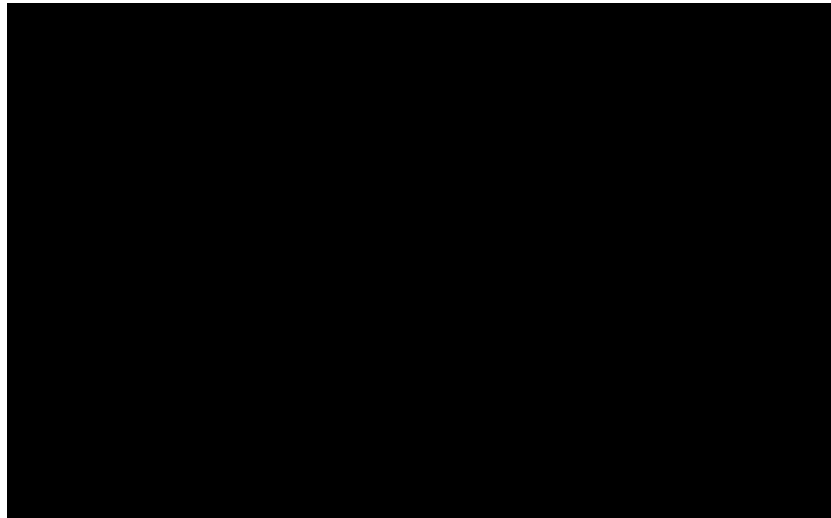


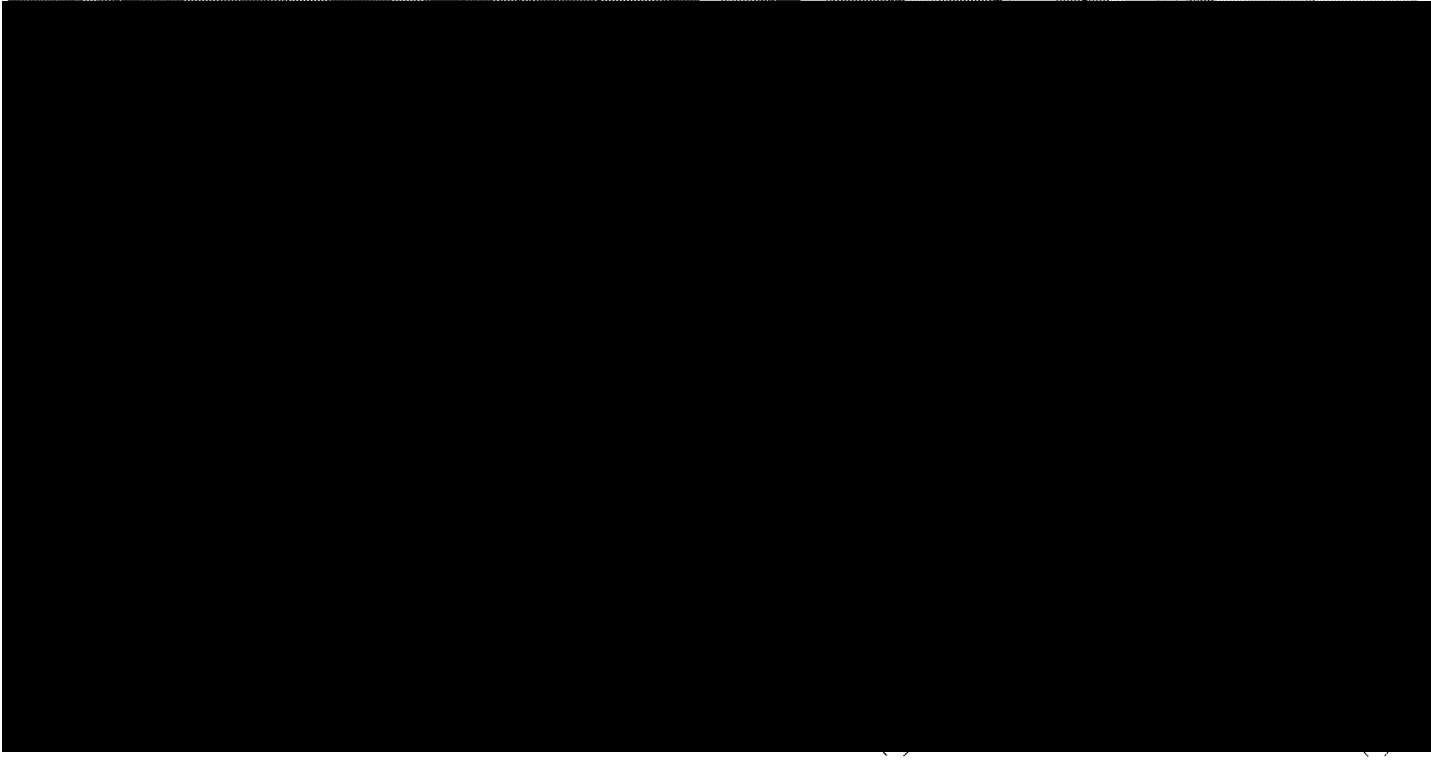
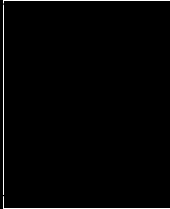
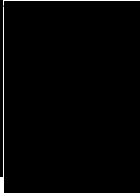
Fig. 36 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (18 and 36  $\mu\text{L/mL}$  of Lipofectin) exposed cells at 1<sup>st</sup> run (Dilution factor 20, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

936-21-M-0643

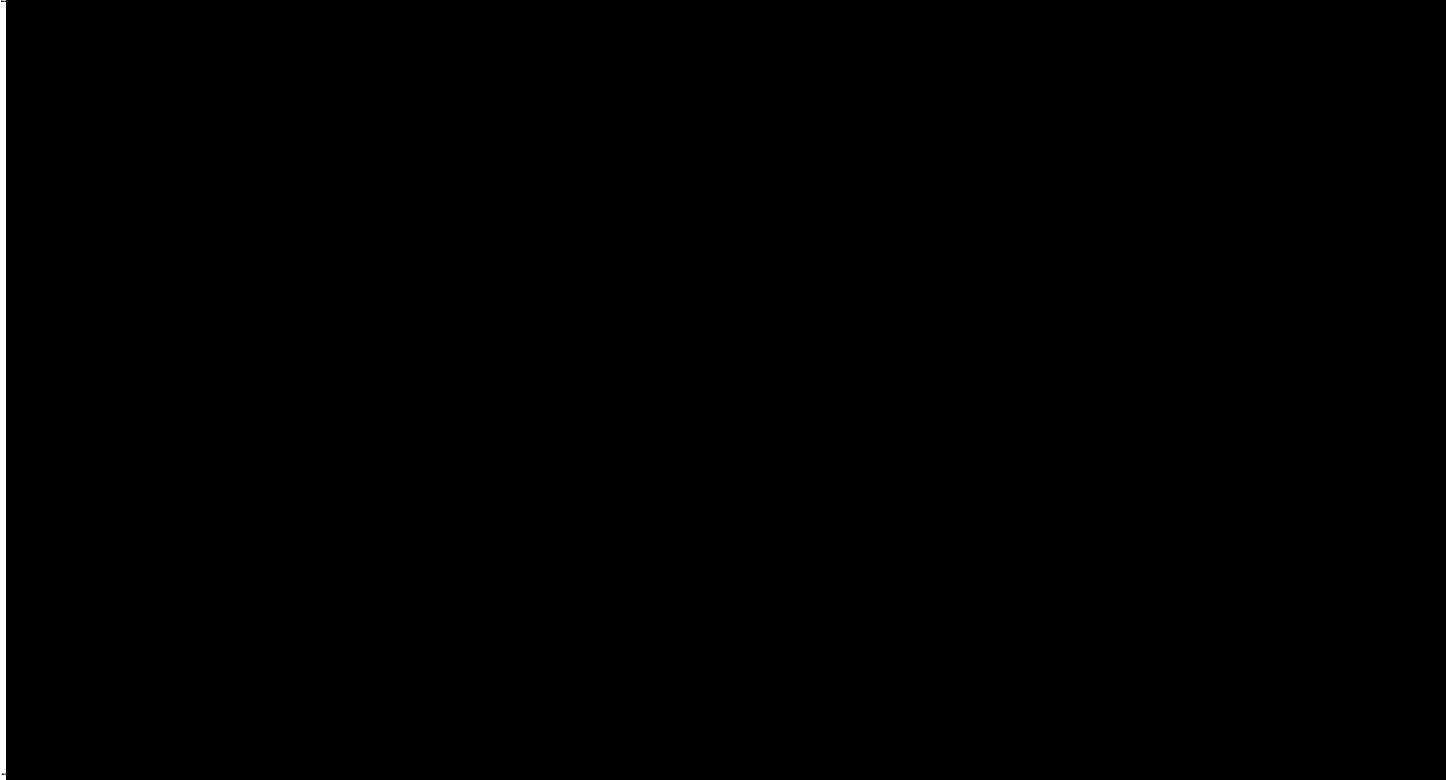


Table 15 Exon skipping efficiency by 4200 TapeStation (ASO-1 exposed cells, 1<sup>st</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0643

Table 16 Exon skipping efficiency by 4200 TapeStation (ASO-3 exposed cells, 1<sup>st</sup> run)

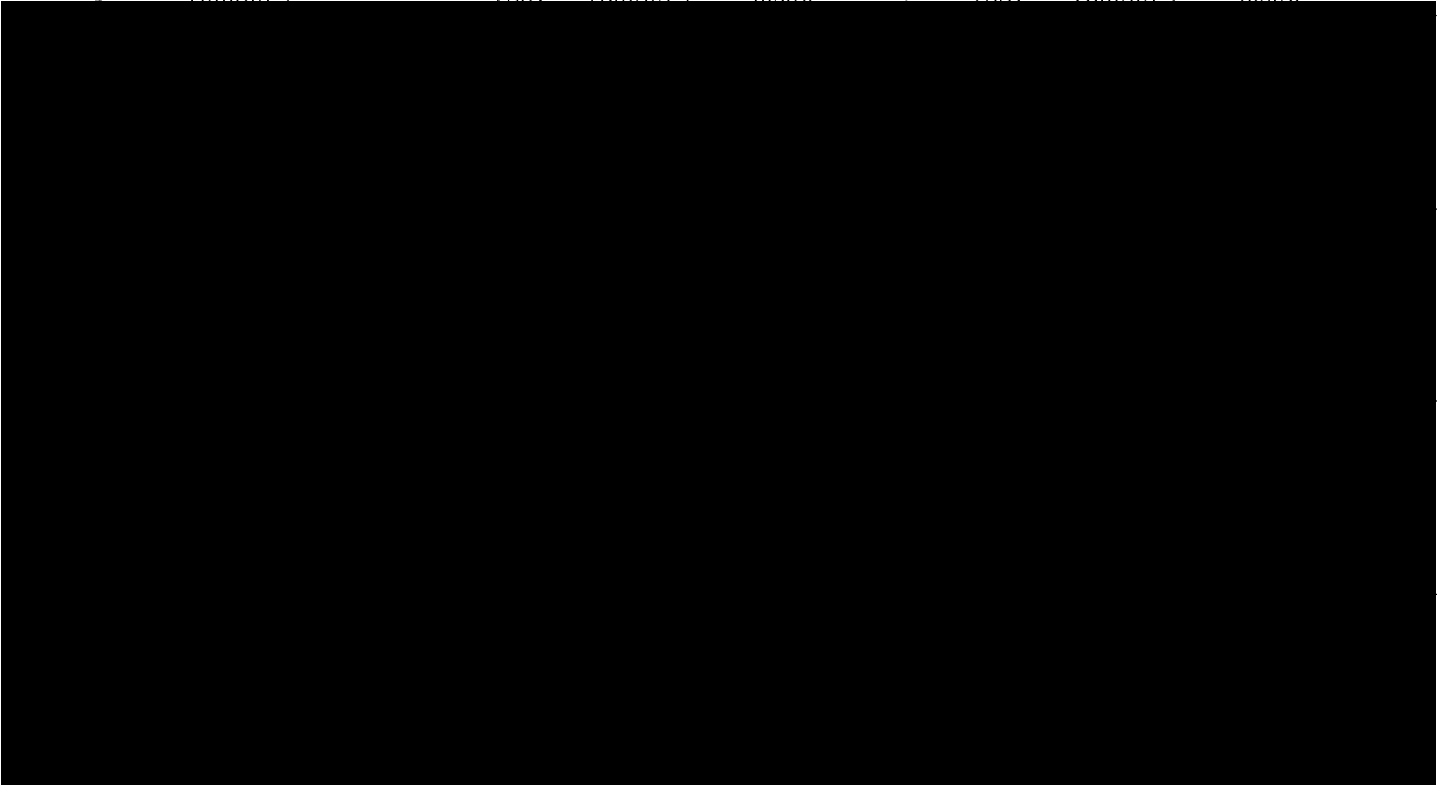
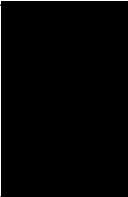

Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable



936-21-M-0643

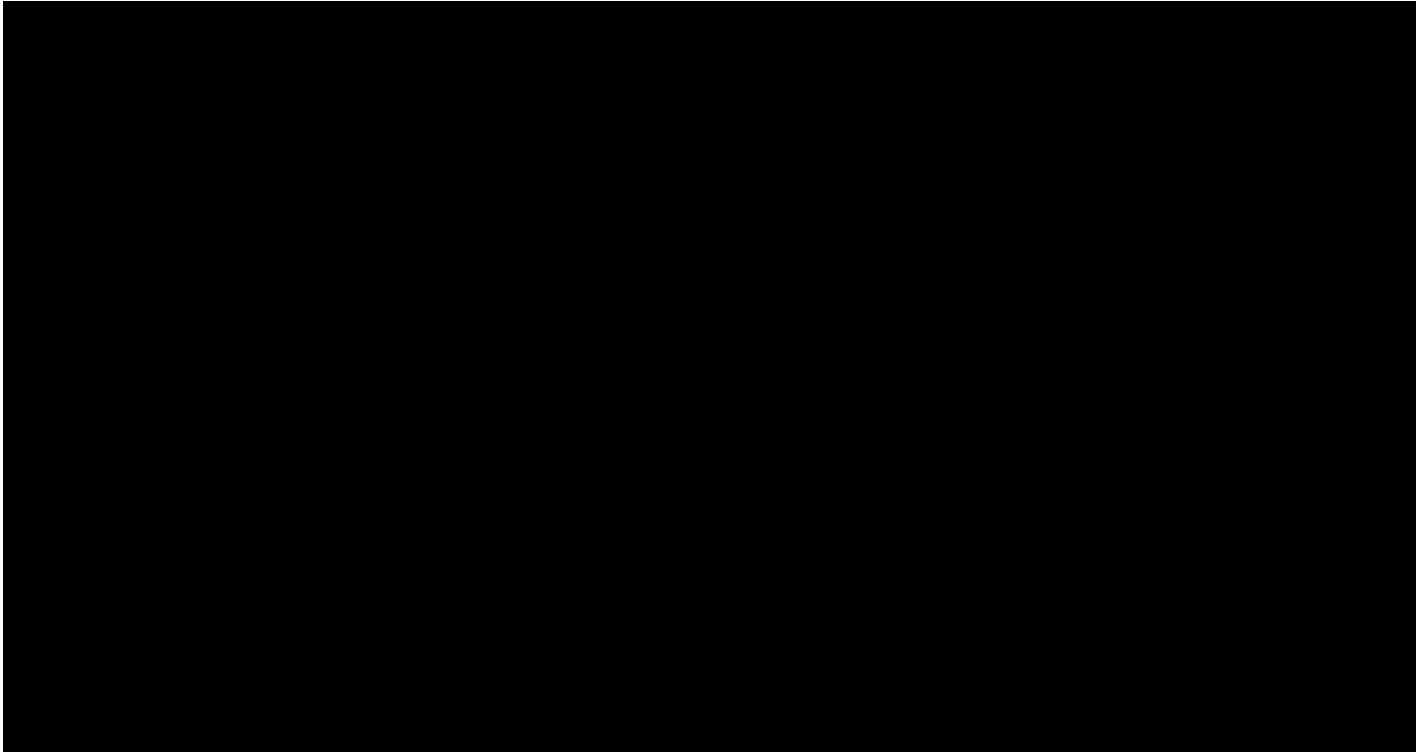





Table 17 Exon skipping efficiency by 4200 TapeStation (ASO-4 exposed cells, 1<sup>st</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment Conc. (nmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (nmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0643

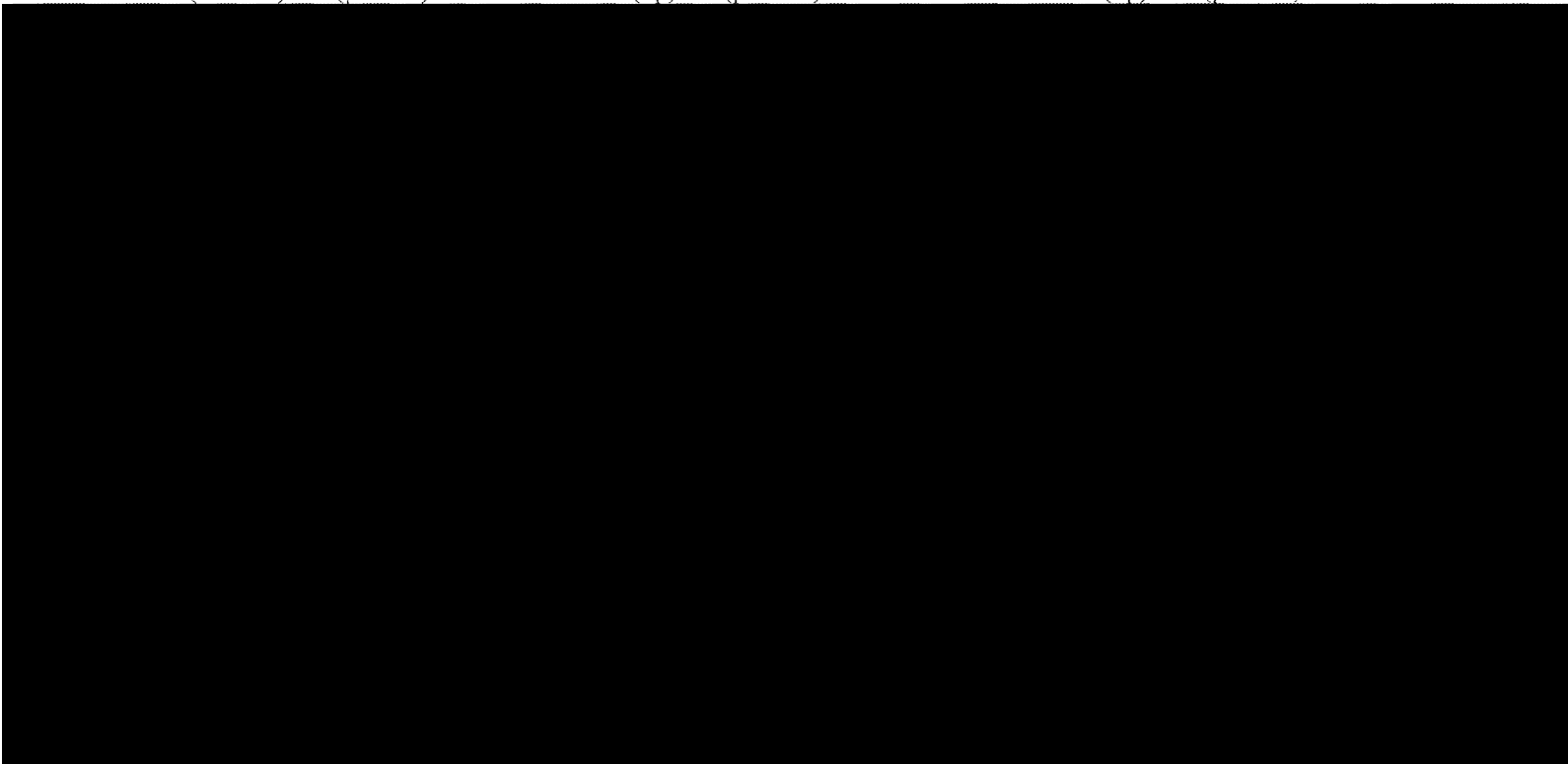






Table 18 Exon skipping efficiency by 4200 TapeStation (ASO-5 exposed cells, 1<sup>st</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											
											N/A
											
											N/A
											N/A
											N/A
											
											
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0643








Table 19 Exon skipping efficiency by 4200 TapeStation (mixture of ASO-3, ASO-4 and ASO-5 exposed cells, 1<sup>st</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	Lipofectin conc. (μL/mL)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
												N/A
												N/A
												N/A
												
												
												
												N/A
												N/A
												N/A
												
												
												

n.d.: not detected, N/A: Not Applicable

936-21-M-0643

Table 20 Exon skipping efficiency by 4200 TapeStation (mixture of ASO-3, ASO-4 and ASO-5 exposed cells, 1<sup>st</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	Lipofectin conc. (μL/mL)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
												N/A
												N/A
												N/A
												
												
												
												N/A
												N/A
												N/A
												
												
												

n.d.: not detected, N/A: Not Applicable

7.2.2 2<sup>nd</sup> run

The PCR product was confirmed using the fragment size by 4200 TapeStation. And then, exon skipping efficiencies were calculated by intensity of the fragments. The exon skipping efficiencies f [REDACTED]

[REDACTED] The exon skipping efficiencies for [REDACTED]

[REDACTED] The exon skipping efficiencies for [REDACTED]

[REDACTED] The exon skipping efficiencies for [REDACTED]

[REDACTED] The exon skipping efficiencies were [REDACTED]

[REDACTED]

[REDACTED] The exon skipping efficiencies were [REDACTED]

[REDACTED]

[REDACTED]

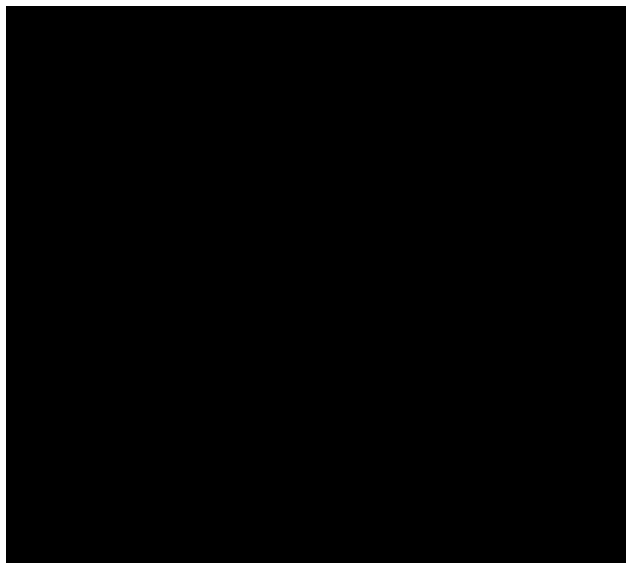


Fig. 37 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-1 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-1 at 300 nmol/L (replicate 1, 2 and 3, respectively)

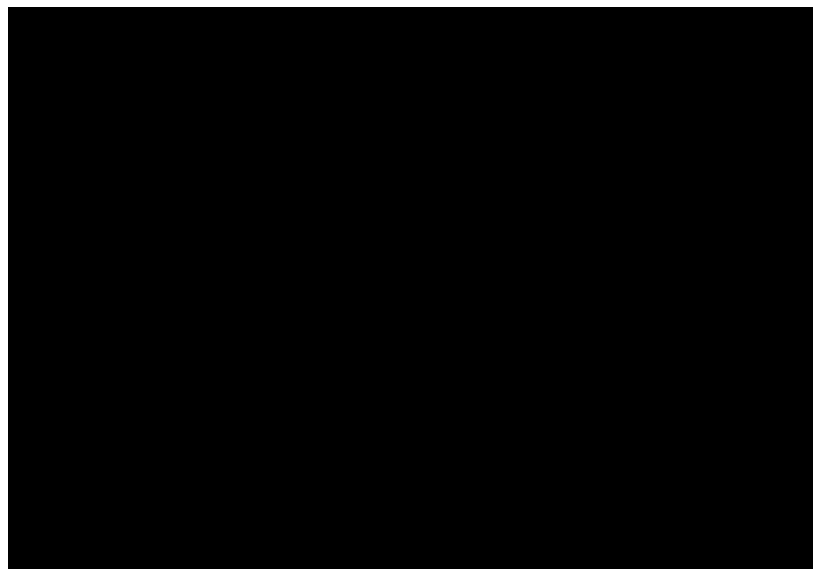


Fig. 38 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-1 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-1 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-1 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

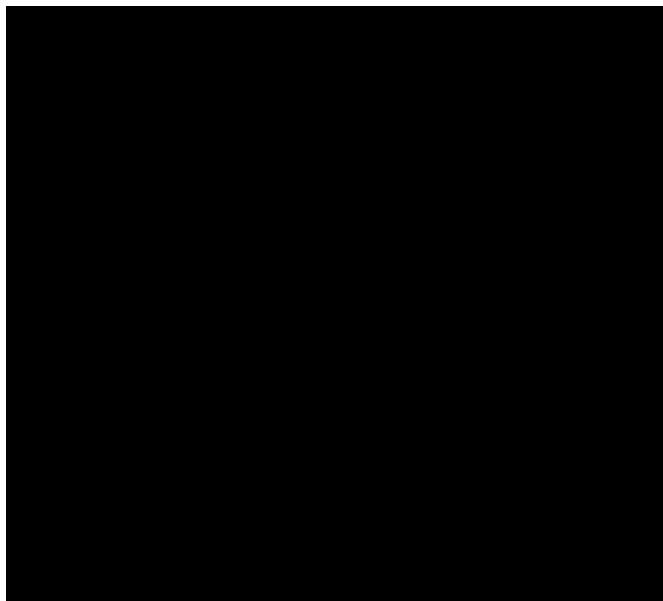


Fig. 39 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-1 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-1 at 600 nmol/L (replicate 1, 2 and 3, respectively)

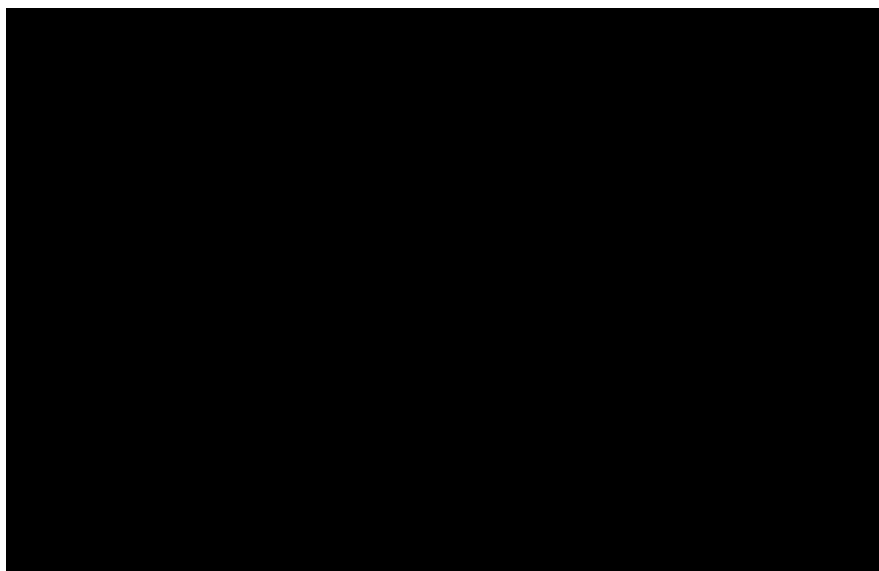


Fig. 40 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-1 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-1 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-1 at 600 nmol/L (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

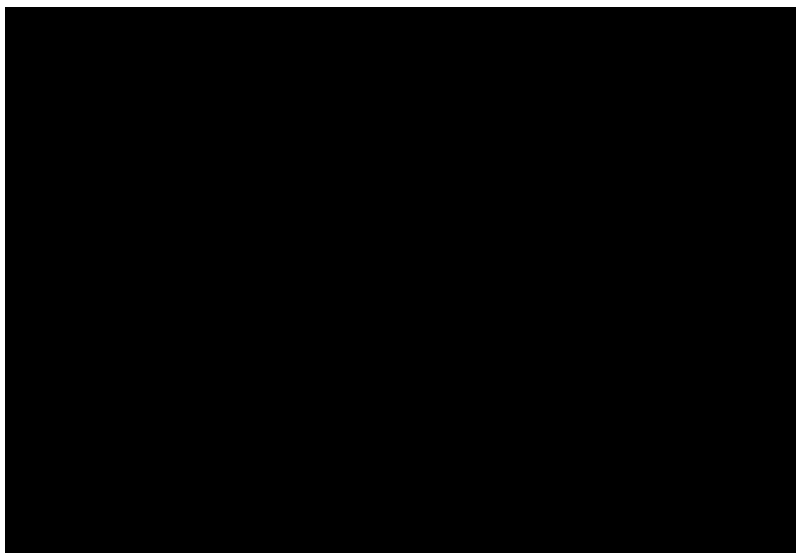


Fig. 41 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-3 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-3 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-3 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

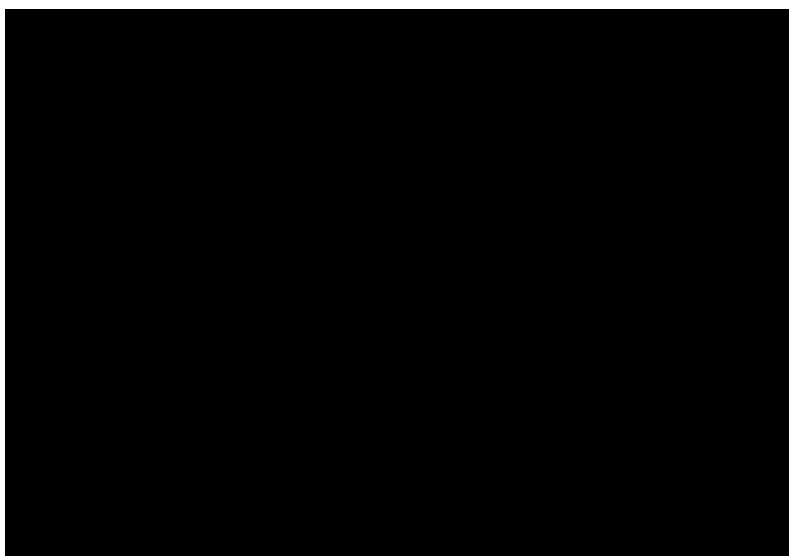


Fig. 42 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-3 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-3 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-3 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.



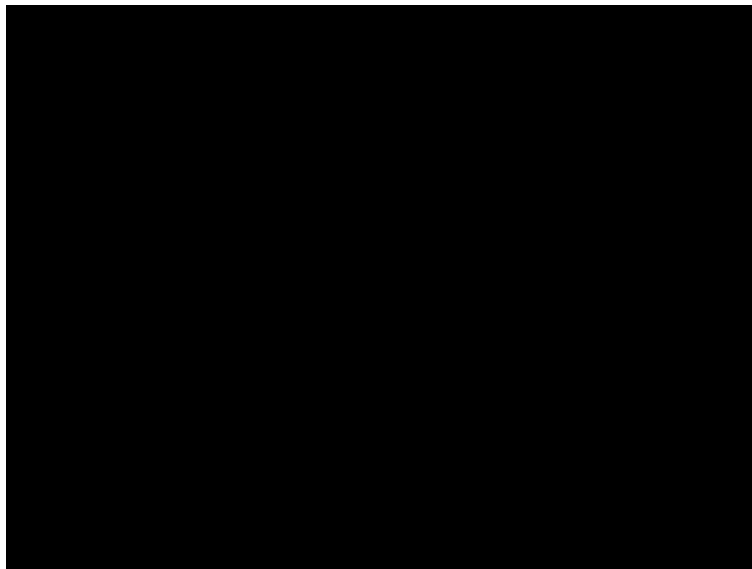


Fig. 43 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-3 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-3 at 600 nmol/L (replicate 1, 2 and 3, respectively)

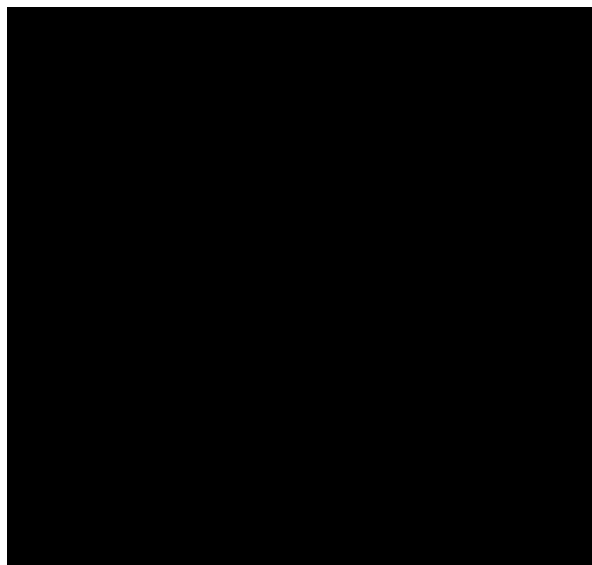


Fig. 44 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-3 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: ASO-3 at 600 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation mark indicate that a noise peak was detected outside upper or lower marker.

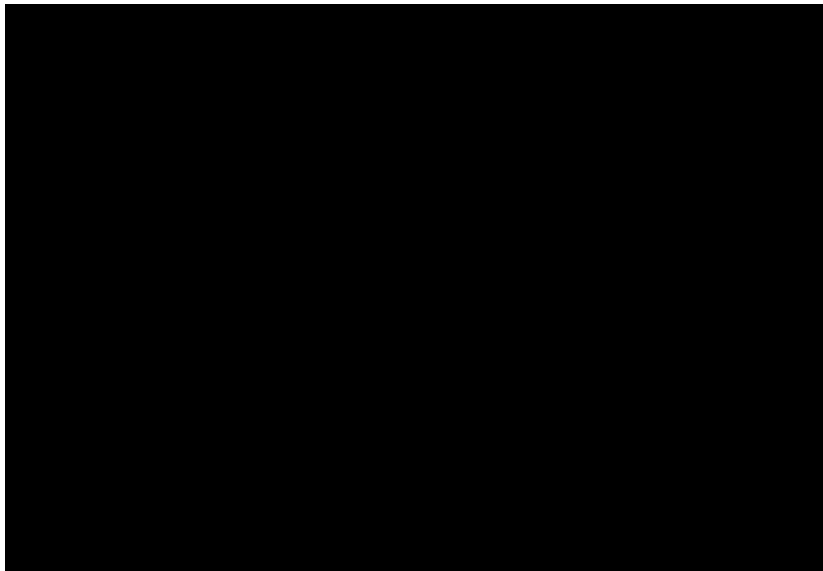


Fig. 45 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-3 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-3 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-3 at 600 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

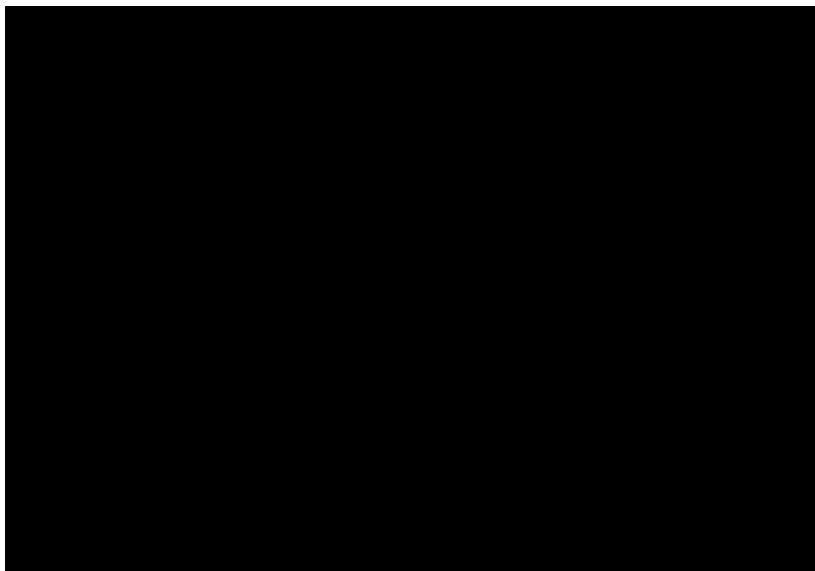


Fig. 46 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively)

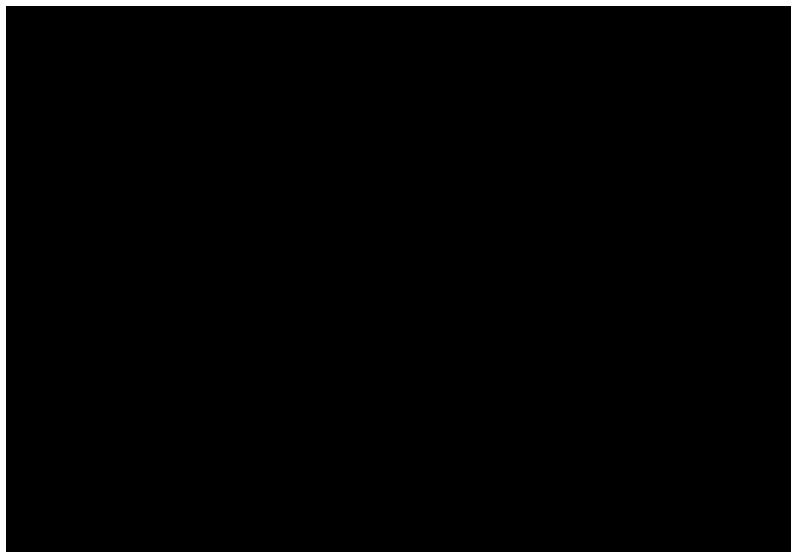


Fig. 47 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

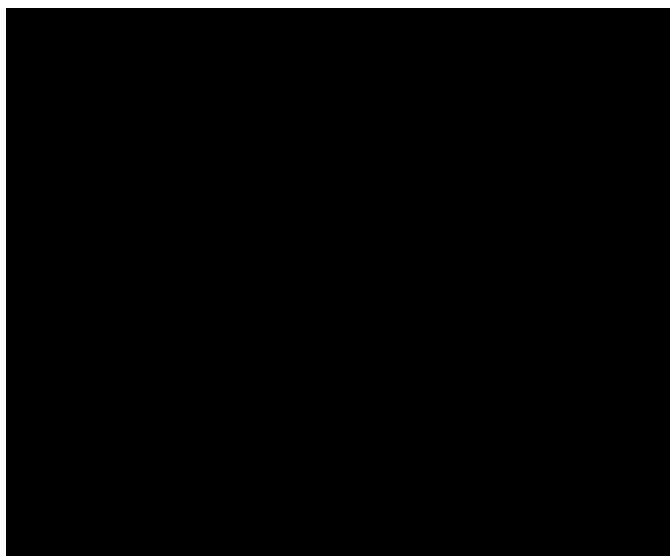


Fig. 48 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 600 nmol/L (replicate 1, 2 and 3, respectively)

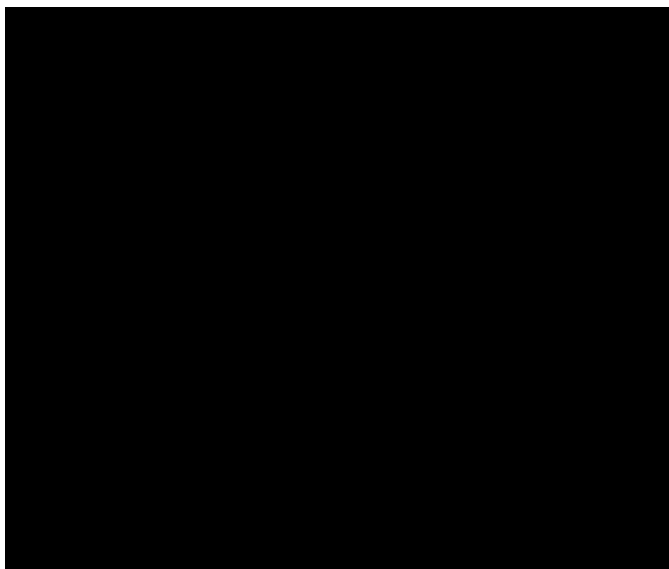


Fig. 49 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control for ASO-4 at 600 nmol/L (replicate 1, 2 and 3, respectively)

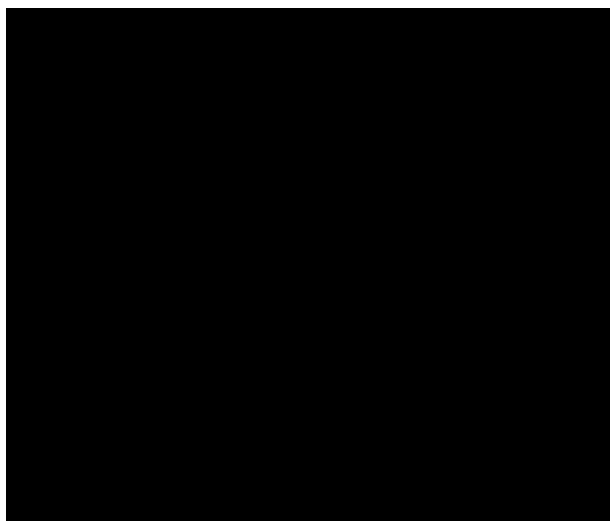


Fig. 50 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-4 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: ASO-4 at 600 nmol/L (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

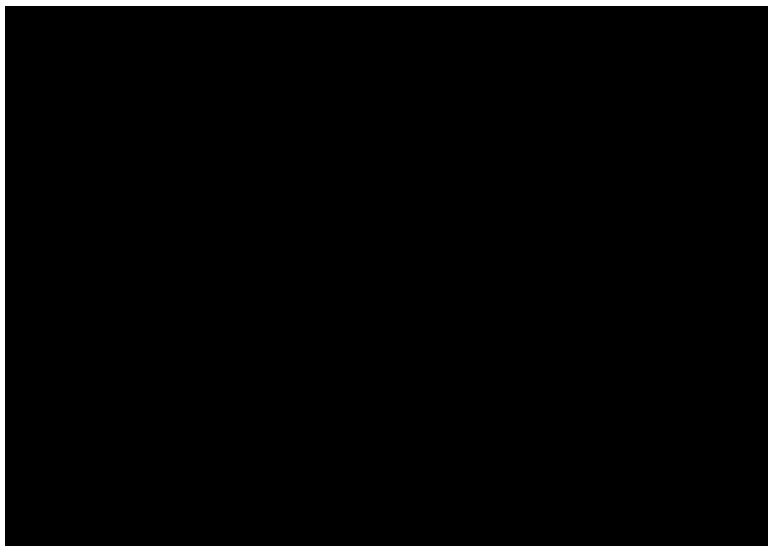


Fig. 51 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

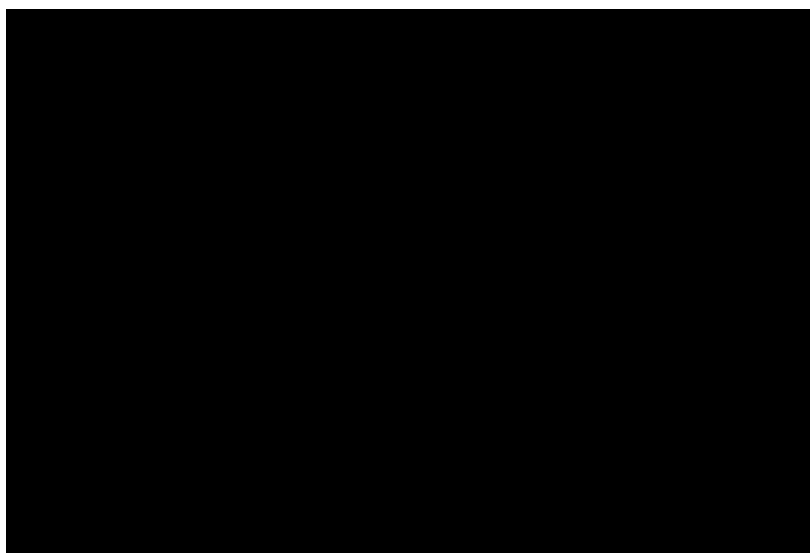


Fig. 52 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-5 at 300 nmol/L (replicate 1, 3 and 2, respectively)

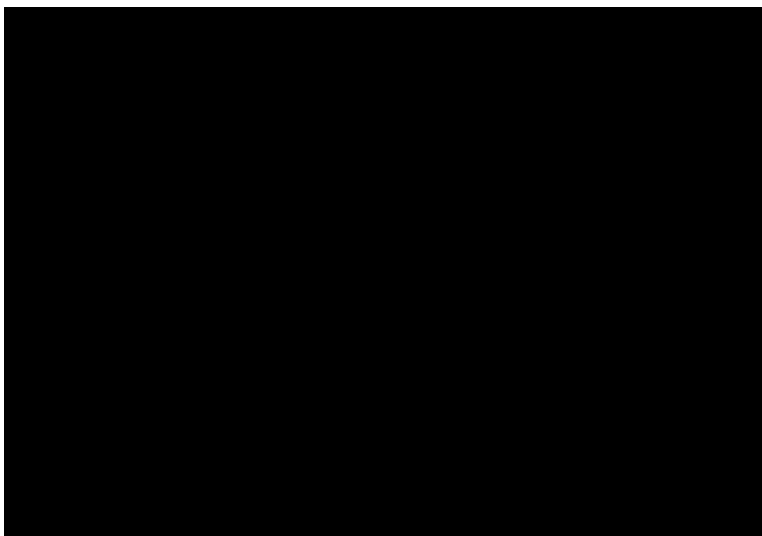


Fig. 53 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

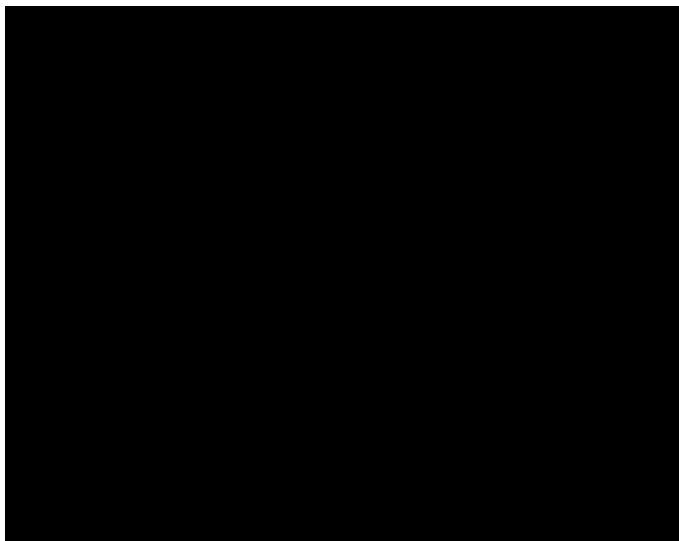


Fig. 54 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

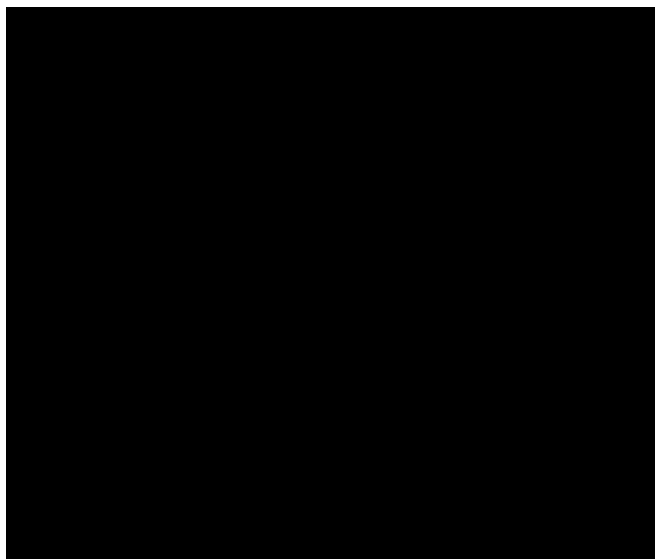


Fig. 55 Electrophoresis by 4200 TapeStation for amplified DMD gene of ASO-5 exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation mark indicate that a noise peak was detected outside upper or lower marker.

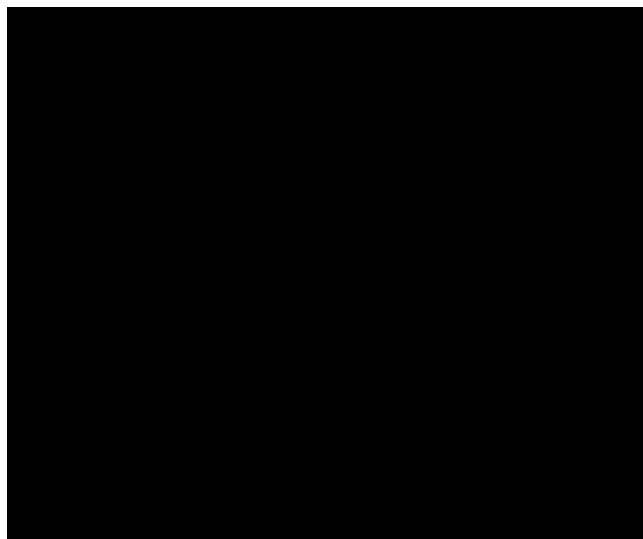


Fig. 56 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (6  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

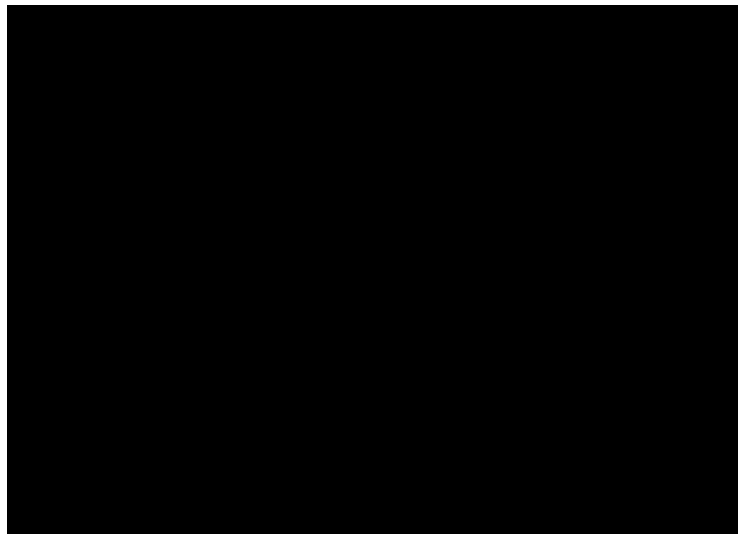


Fig. 57 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (6  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

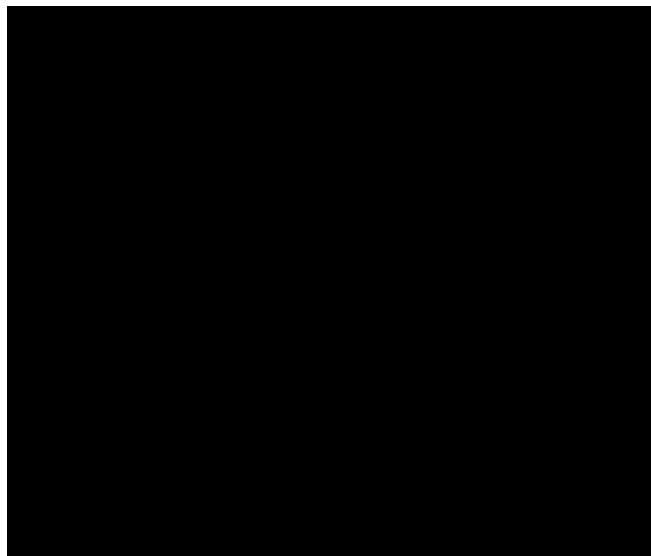


Fig. 58 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (6  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 20, approximately 365 bp and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.



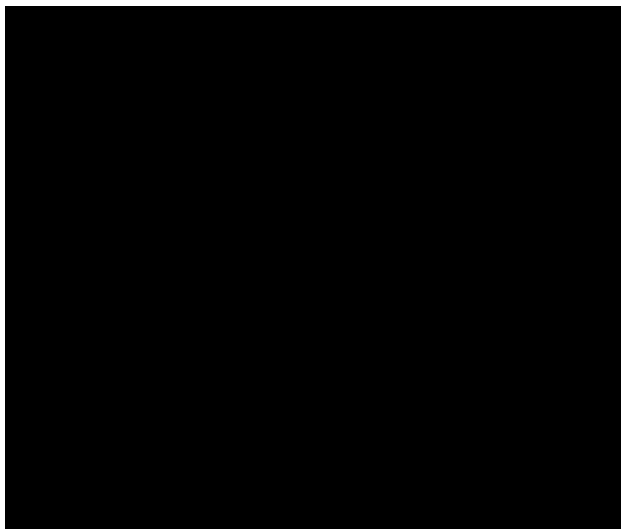


Fig. 59 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (12  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

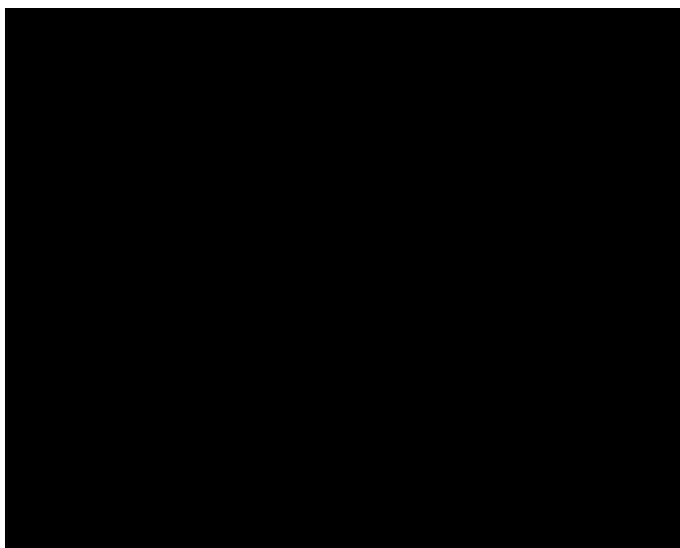


Fig. 60 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (12  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

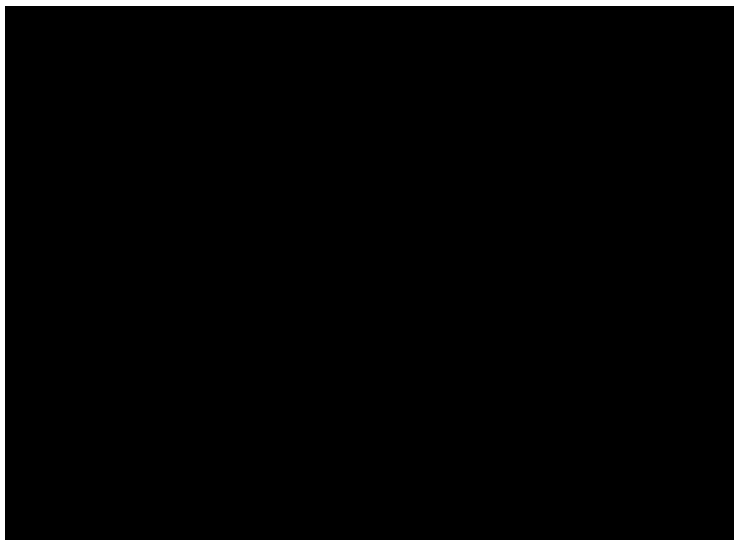


Fig. 61 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (12  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 20, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

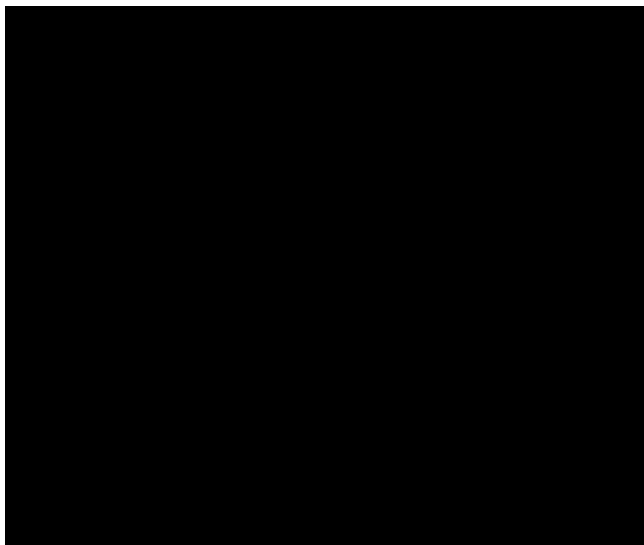


Fig. 62 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (18  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

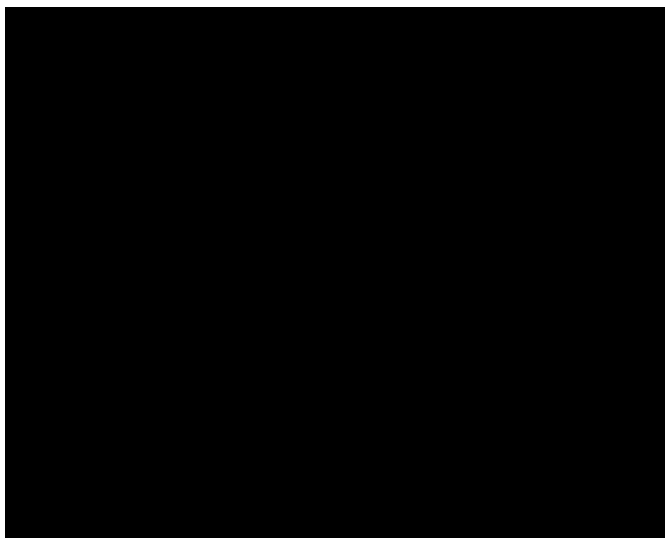


Fig. 63 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (18  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

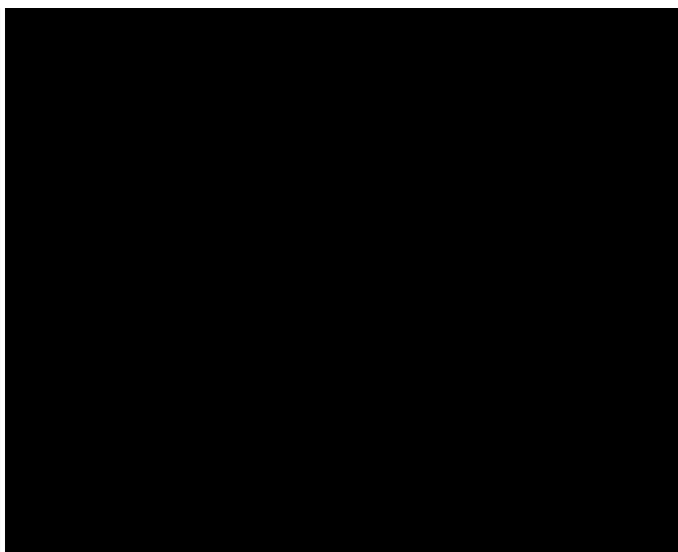


Fig. 64 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (18  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 20, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: mixture of ASO-3, ASO-4 and ASO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

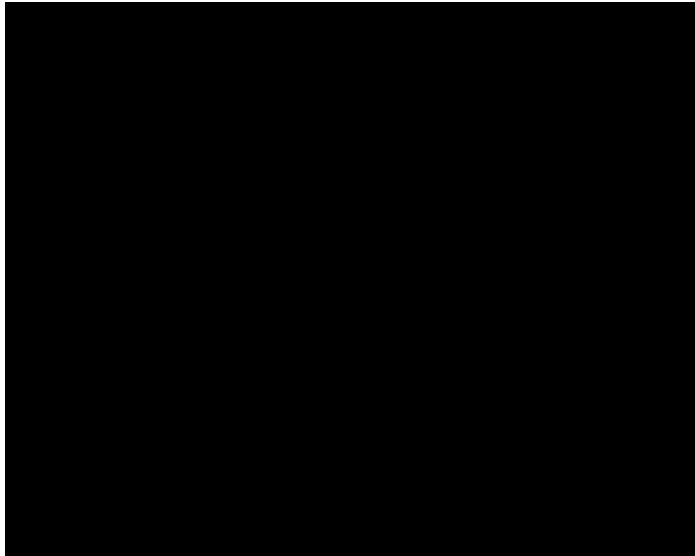


Fig. 65 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (36  $\mu\text{L/mL}$  of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

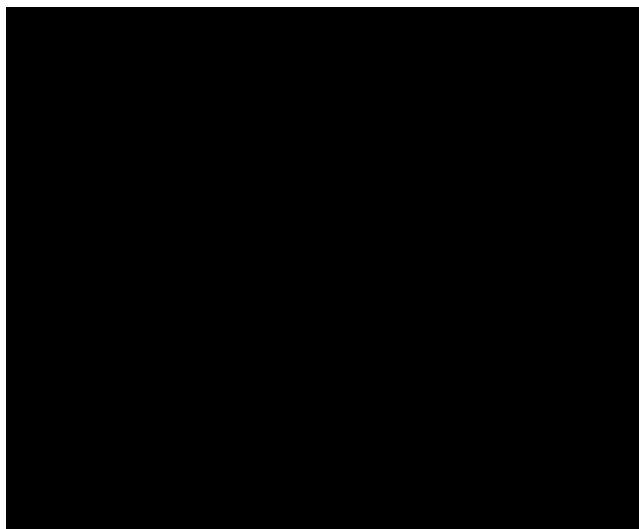


Fig. 66 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (36  $\mu\text{L/mL}$  of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 100, approximately 365 bp and fragment)

A1: Ladder Marker, B1, C1 and D1: control for mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

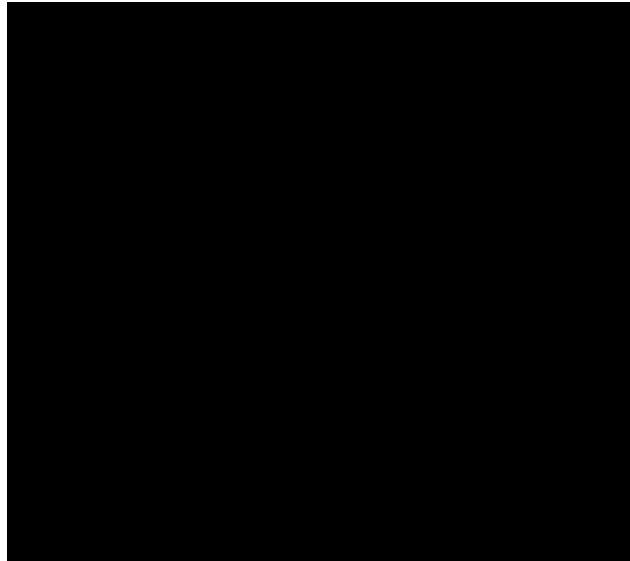


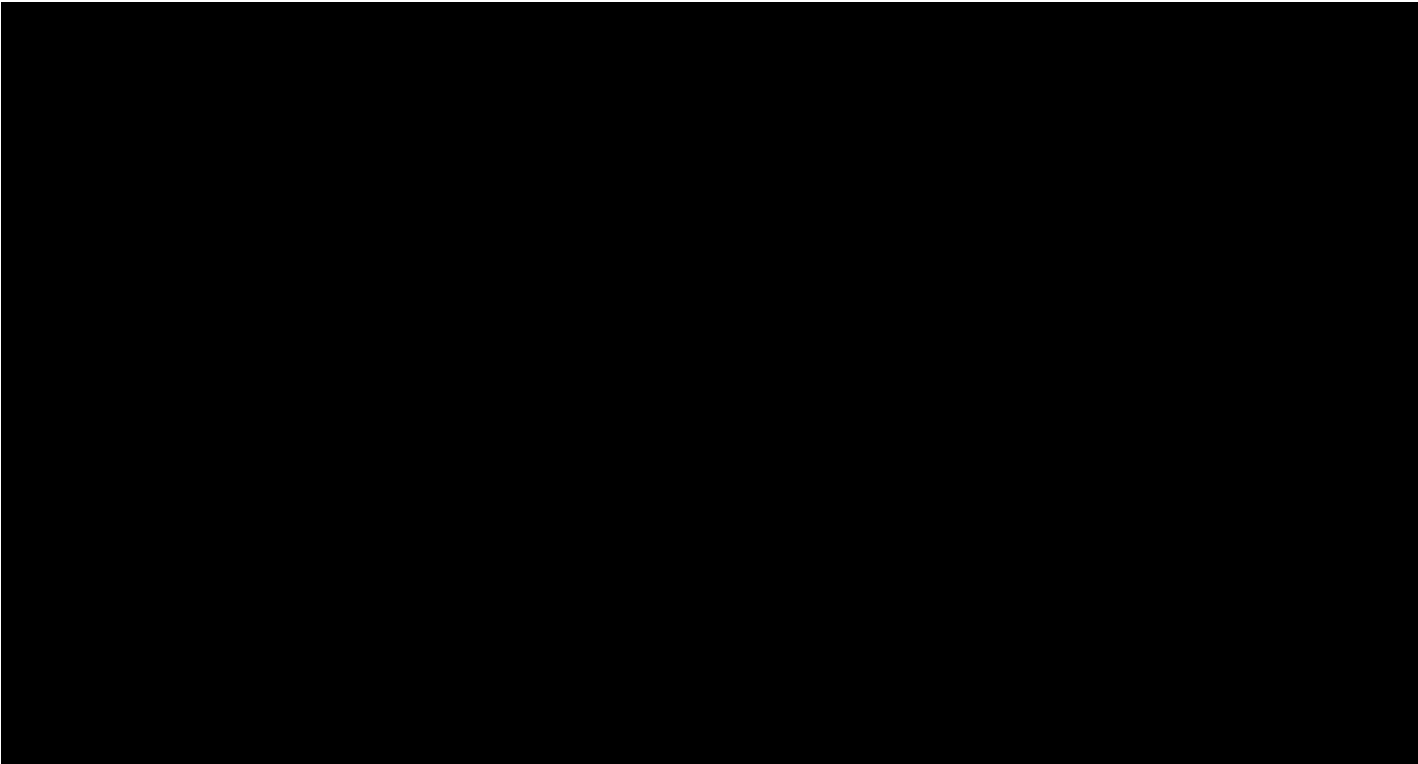

Fig. 67 Electrophoresis by 4200 TapeStation for amplified DMD gene of mixture of ASO-3, ASO-4 and ASO-5 (36  $\mu$ L/mL of Lipofectin) exposed cells at 2<sup>nd</sup> run (Dilution factor 20, approximately 365 bp and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: mixture of ASO-3, ASO-4 and ASO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

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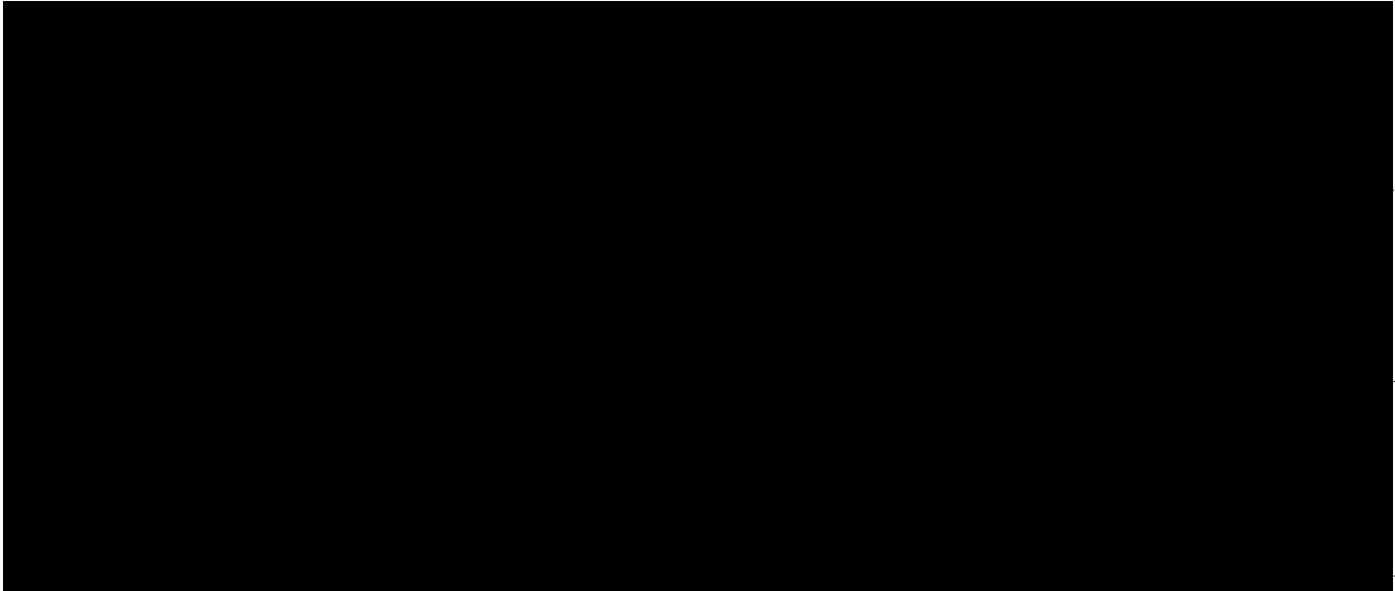
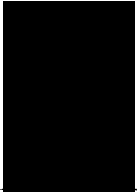
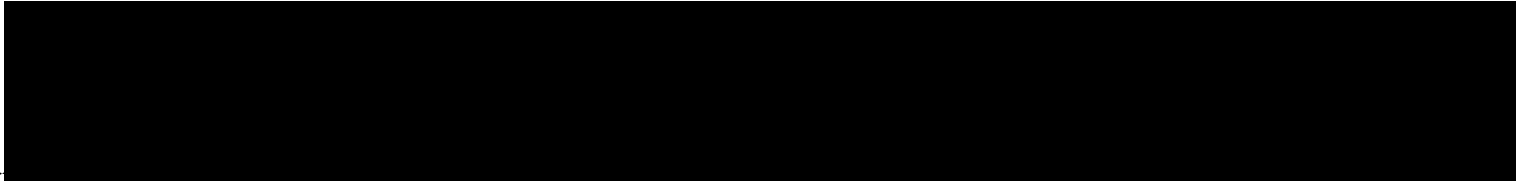
Table 21 Exon skipping efficiency by 4200 TapeStation (ASO-1 exposed cells, 2<sup>nd</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment		Fig.	Size (bp)	Skipped fragment		Fig.	Skipping Efficiency
			Conc. (pmol/L)	Dilution factor	Conc. (pmol/L)			Dilution factor			
											N/A
											N/A
											N/A
											
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-21-M-0643

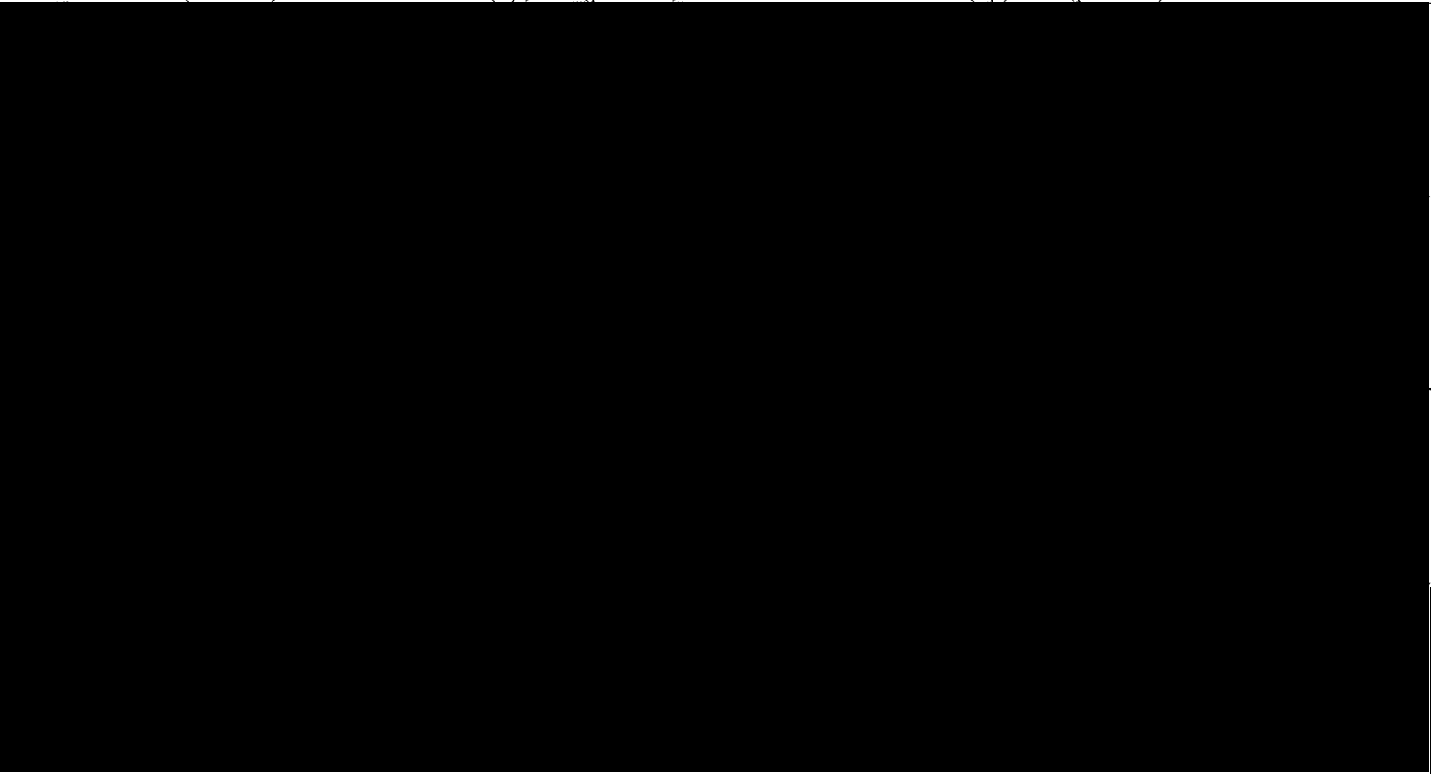


Table 22 Exon skipping efficiency by 4200 TapeStation (ASO-3 exposed cells, 2<sup>nd</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

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Table 23 Exon skipping efficiency by 4200 TapeStation (ASO-4 exposed cells, 2<sup>nd</sup> run)

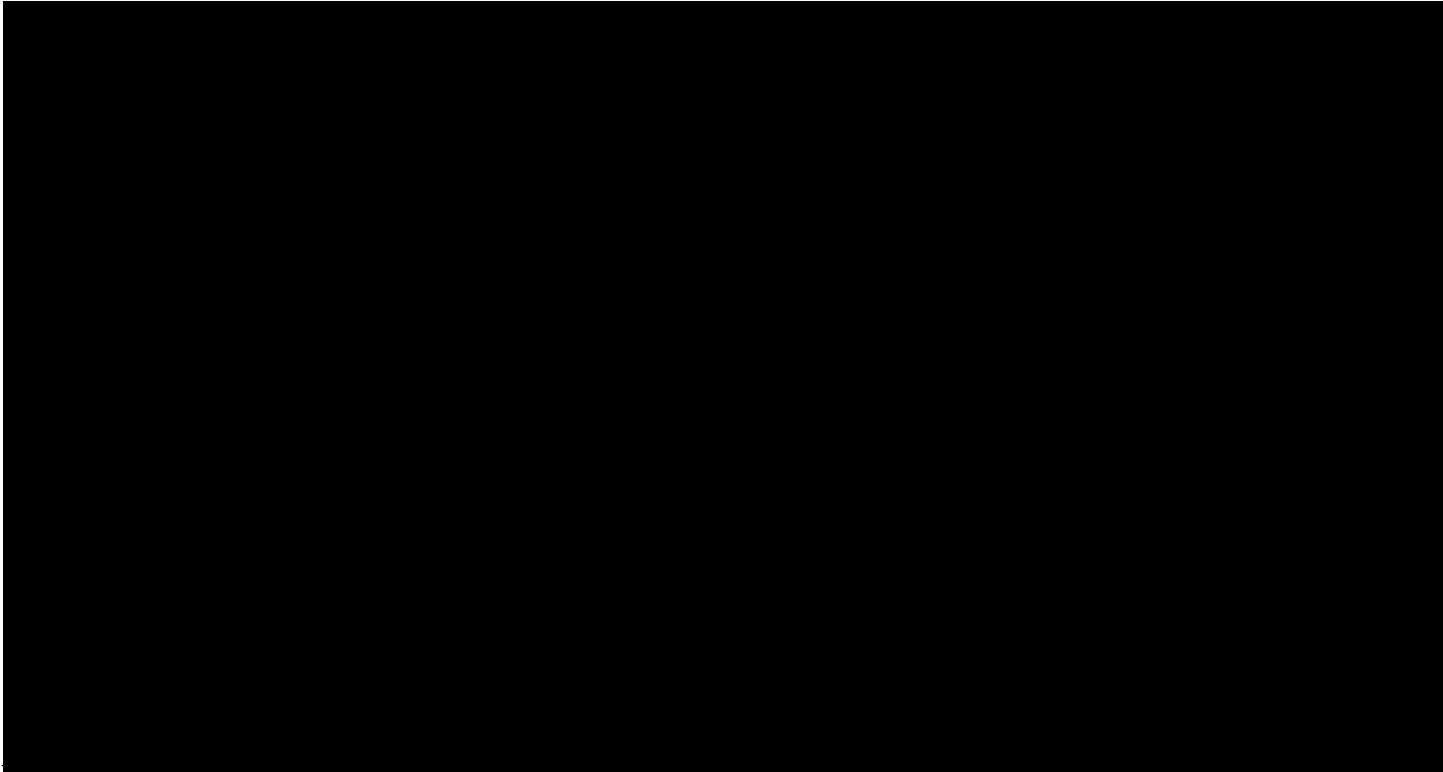
Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable



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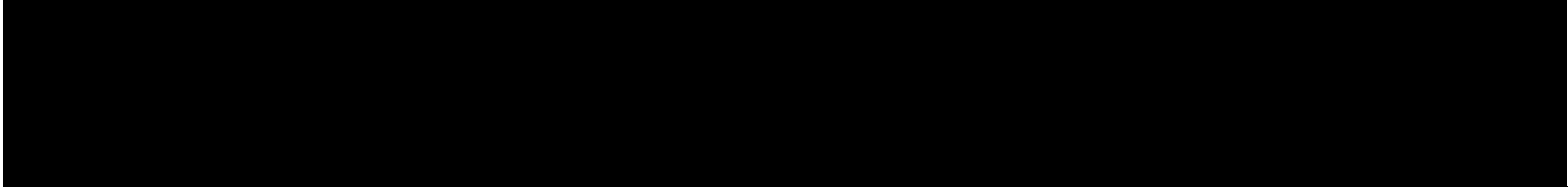
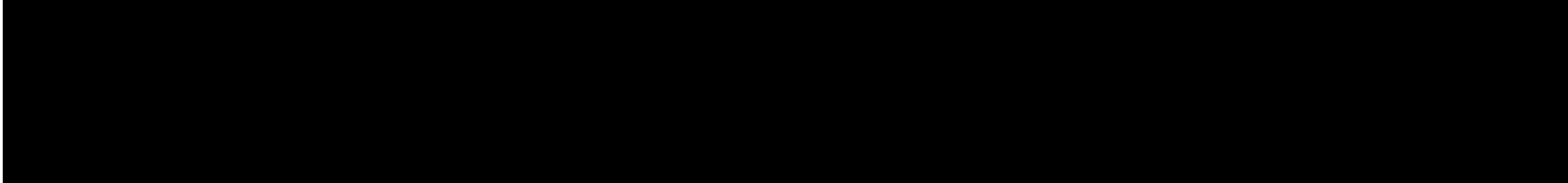
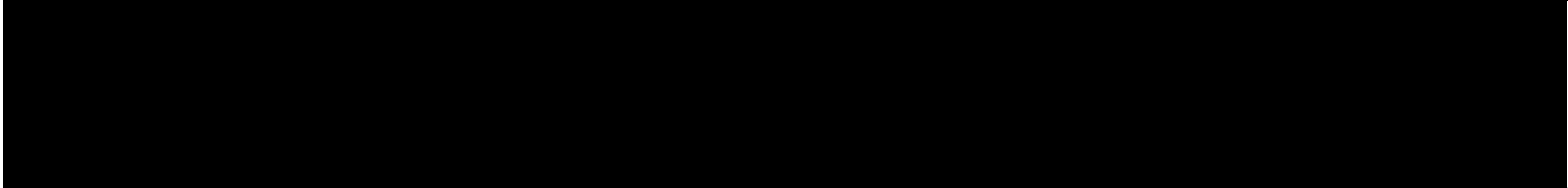
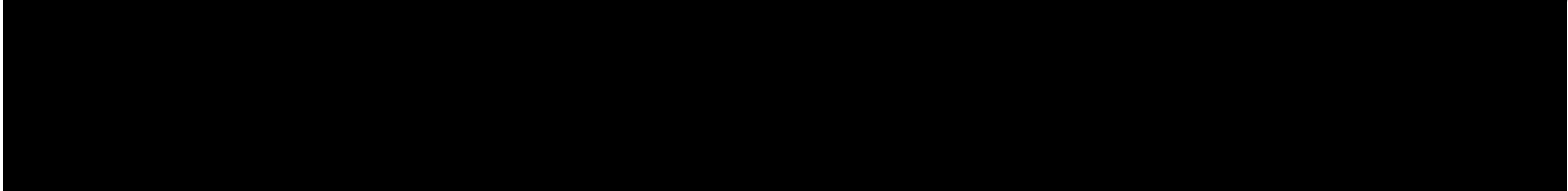
Table 24 Exon skipping efficiency by 4200 TapeStation (ASO-5 exposed cells, 2<sup>nd</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-21-M-0643

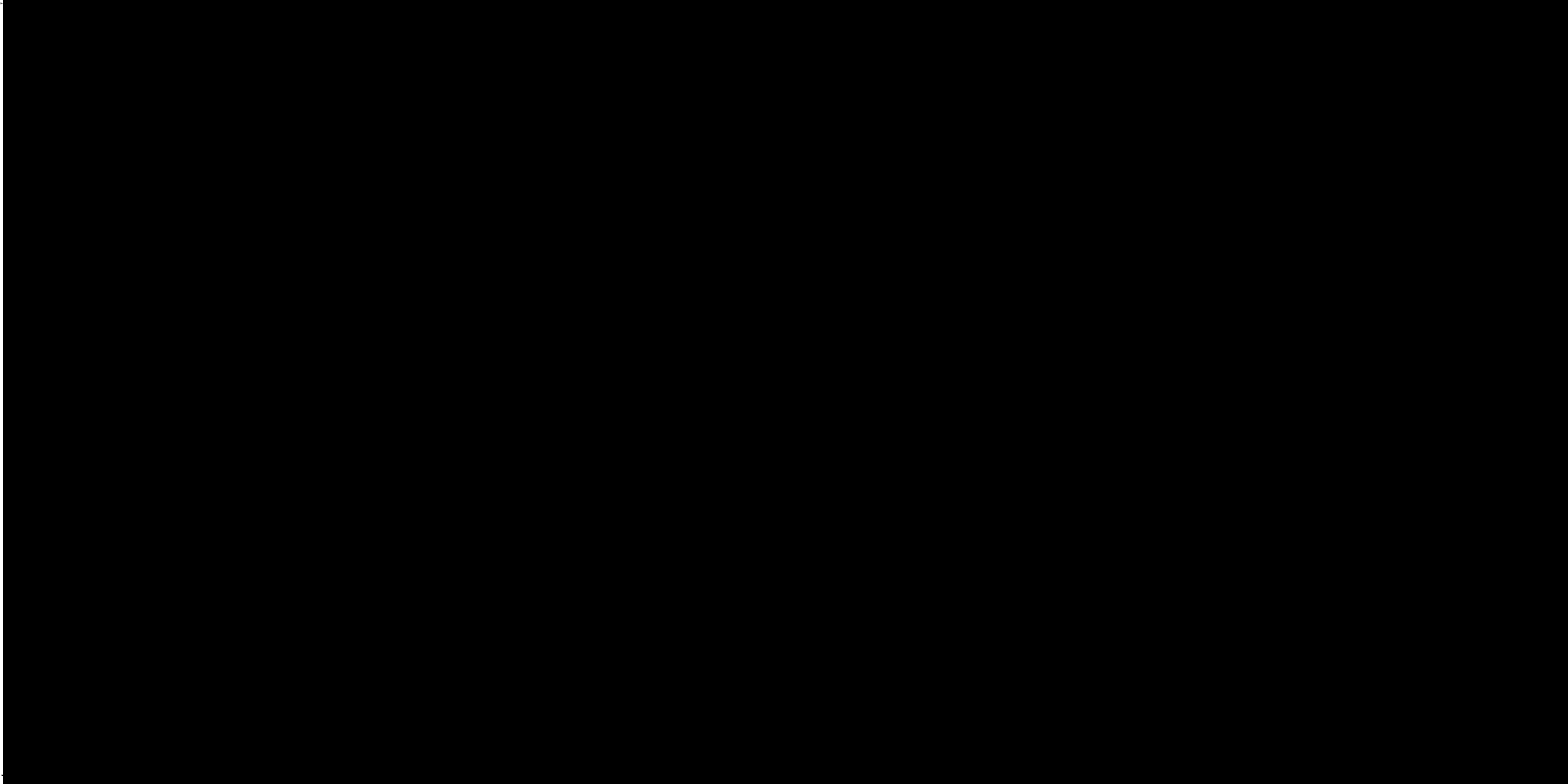
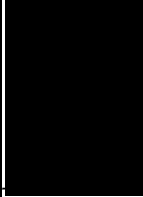
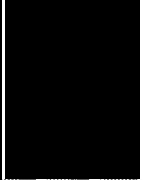
Table 25 Exon skipping efficiency by 4200 TapeStation (mixture of ASO-3, ASO-4 and ASO-5 exposed cells, 2<sup>nd</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	Lipofectin conc. (μL/mL)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
												N/A
												N/A
												N/A
												N/A
												N/A
												N/A
												N/A
												N/A
												N/A
												N/A
												N/A
												N/A

n.d.: not detected, N/A: Not Applicable

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Table 26 Exon skipping efficiency by 4200 TapeStation (mixture of ASO-3, ASO-4 and ASO-5 exposed cells, 2<sup>nd</sup> run)

Exposure Sample	Exposure conc. (nmol/L)	Lipofectin conc. (uL/mL)	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
												N/A
												N/A
												N/A
												
												N/A
												N/A
												N/A
												

n.d.: not detected, N/A: Not Applicable

8. STORAGE OF STUDY PLAN AND FINAL REPORT

The original study plan and final report are one copy and stored in the archives room (4207) of the testing facility. The duplicates of study plan and final report as PDF files are sent to the sponsor.

9. STORAGE AND RETENTION OF DOCUMENT AND RECORD

The original study plan, the original study plan amendment, the original final report, and other records concerning the study were stored in the archives room (4207) of the testing facility for 5 years after the study completion date. The test substances were stored in the archives room (4207) of the testing facility for 1 year after the study completion date. The management (disposal or return) of these items after the storage period will be notified the sponsor in writing before the completion of the storage period. After the storage period, a notice will be given in writing to the sponsor and the stored materials will be returned or disposed.

# Exhibit B



Study Number	936-21-M-0644
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## FINAL REPORT

The assessment of exon skipping activities by PMO in myotube using Endo-Porter DMSO as a transfection reagent.

May 2023

Chemicals assessment and research center  
Chemicals Evaluation and Research Institute, Japan (CERI)

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1. TITLE

The assessment of exon skipping activities by PMO in myotube using Endo-Porter DMSO as a transfection reagent.

2. STUDY NUMBER

936-21-M-0644

3. OBJECTIVE

To assess exon skipping activities of sixteen different PMO in myotube.

4. TESTING FACILITY

Name: Chemicals Assessment and Research Center, Chemicals Evaluation and Research Institute, Japan (CERI)

Address: 1600, Shimotakano, Sugito-machi, Kitakatsushika-gun, Saitama 345-0043, Japan

5. PERIOD OF STUDY

Study initiation date.: March 25, 2022

Experimental starting date.: March 31, 2022

Experimental completion date.: June 9, 2022

Study completion date.: May 17, 2023



### 6.1.1 Test substances

Table 1 Information of test substances

[illegible]

Test substance ID	PMO-6	PMO-7	PMO-8	PMO-9
				

Test substance ID	PMO-10	PMO-11	PMO-12	PMO-13

Test substance ID	PMO-14	PMO-15	PMO-16	PMO-17
				

Gloves, a mask and a lab coat were worn when handling these substances.

6.1.2 Vehicle control substance

(1) Name

Water for injection

(2) Grade

Japanese Pharmacopoeia

(3) Lot number

K9K87

(4) Manufacturer

Otsuka Pharmaceutical Factory

(5) Storage condition

Vehicle was stored at room temperature.

(6) Handling precautions

Gloves, a mask, and a lab coat were worn when handling.

6.2 Cells

Name: [REDACTED]

Supplier: [REDACTED]  
[REDACTED]

Lot number: [REDACTED]

6.3 Reagents, equipments and softwares

(1) Reagents

- PBS (-) powder (Thermo Fisher Scientific)
- SkGM™-2 BulletKit™ (Lonza)
- Trypsin (Thermo Fisher Scientific)
- Dulbecco's Modified Eagle Medium: F12 (DMEM: F12) (Lonza)
- Horse serum (Thermo Fisher Scientific)
- Trypan Blue Stain 0.4% (Thermo Fisher Scientific)
- Endo-Porter, DMSO (GENE TOOLS)
- Trizol (Thermo Fisher Scientific)
- 2-Propanol (Molecular Biology, FUJIFILM Wako Pure Chemical)
- Ethanol (Guaranteed Reagent, FUJIFILM Wako Pure Chemical)
- Chloroform (Guaranteed Reagent, FUJIFILM Wako Pure Chemical)
- Water for injection (Grade: Japanese Pharmacopoeia, Otsuka Pharmaceutical Factory)
- QIAshredder (QIAGEN)
- RNeasy PLUS Micro Kit (QIAGEN)
- High Sensitivity RNA ScreenTape (Agilent)
- High Sensitivity RNA Sample Buffer (Agilent)

- High Sensitivity RNA Ladder (Agilent)
- Nuclease Free Water (Thermo Fisher Scientific)
- Titan One-tube RT-PCR system (Sigma-Aldrich)
- Outer Forward Primer (PS192: 5'-CTT GGA CAG AAC TTA CCG ACT GG-3')
- Outer Reverse Primer (PS197: 5'-GTT TCT TCC AAA GCA GCC TCT CG-3')
- Inner Forward Primer (PS193: 5'-GCA GGA TTT GGA ACA GAG GCG-3')
- Inner Reverse Primer (PS195: 5'-CAT CTA CAT TTG TCT GCC ACT GG-3')
- Tth DNA Polymerase (Sigma-Aldrich)
- PCR Nucleotide Mix (Sigma-Aldrich)
- High Sensitivity D1000 ScreenTape (Agilent)
- High Sensitivity D1000 Sample Buffer (Agilent)
- High Sensitivity D1000 Ladder (Agilent)
- TE Buffer, 1X (Molecular Biology Grade, Promega)

(2) Equipments

- CO<sub>2</sub> incubator (SANYO, MCO-175M)
- Biosafety cabinet (DALTON, NSC-II-A-1200)
- Water bath (Yamato, Thermo-Mate BF400)
- Refrigerated centrifuge (Kubota, 3740)
- Ultrapure water system (Komatsu Electronics, KE0147UC)
- Magnetic stirrer (ADVANTEC, SR500)
- Refrigerator (SANYO, MPR-514 and Panasonic, MPR-162DCN-PJ)
- Freezer (SANYO, MDF-U537D, SANYO, MDF-U332 and SANYO, MDF-U333)
- Deep freezer (Panasonic, MDF-U500VXS5-PJ)
- Automated RNA purification system (QIAGEN, QIAcube)
- Liquid nitrogen tank (CRYOGENIC EQUIPMENT, SR-31)
- NanoDrop One (Thermo Fisher Scientific, ND-ONE-W)
- Micro refrigerated centrifuge (TOMY, MX-205)
- TapeStation (Agilent, 4200 TapeStation)
- Thermal cycler (ProFlex, Thermo Fisher Scientific)
- Dry bath incubator (BSR-MK10, Bio medical science)

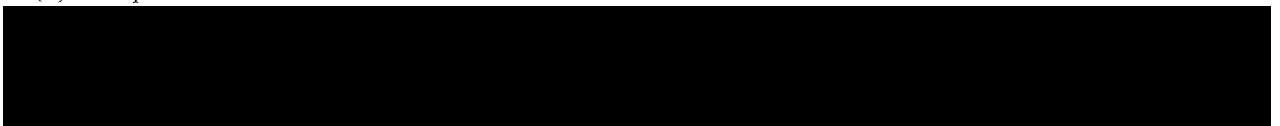
(3) Software

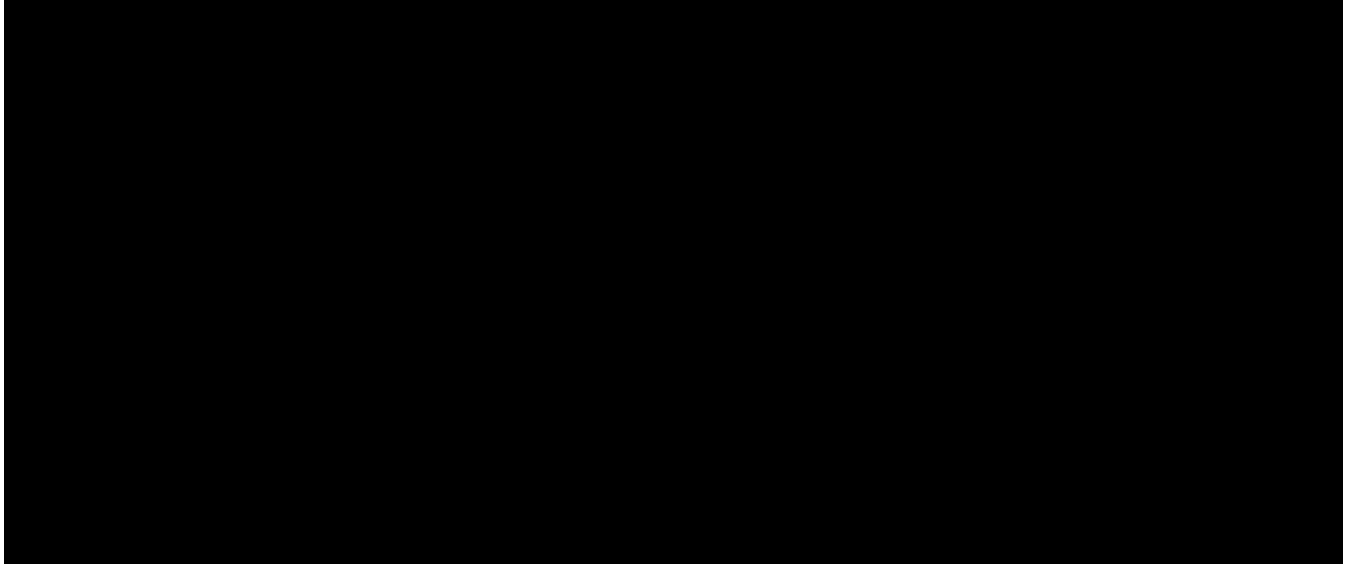
- Excel 2019 (Microsoft)

6.4 Culture medium

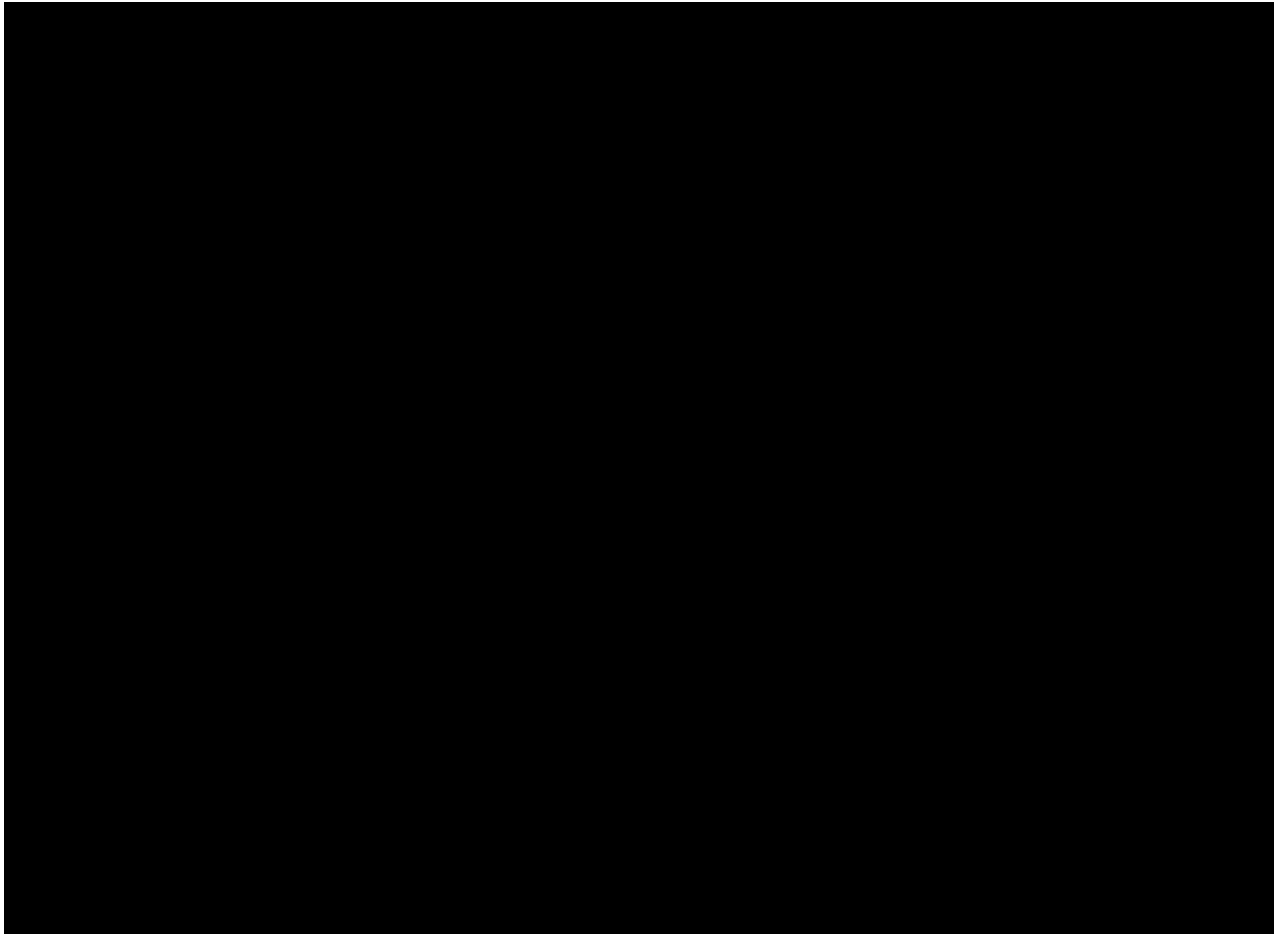
- Primary culture medium (SkGM™-2)

(1) Composition

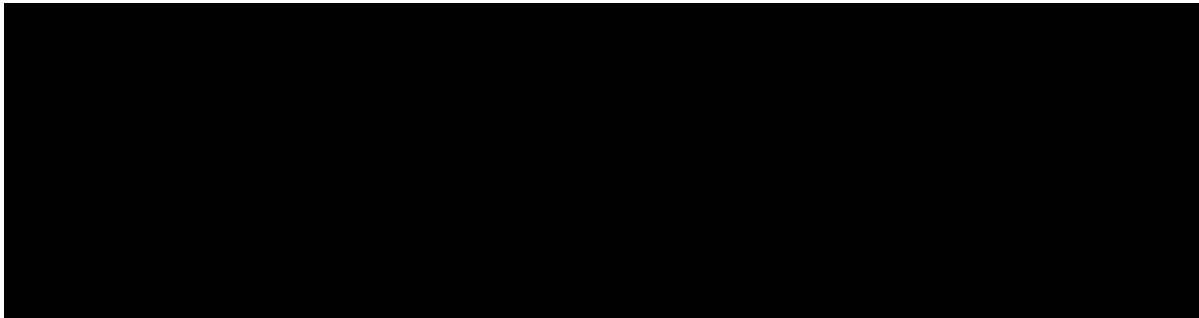




6.5 Cell culture and differentiation



6.6 Transfection



6.6.2 Preparation of medium containing test substance

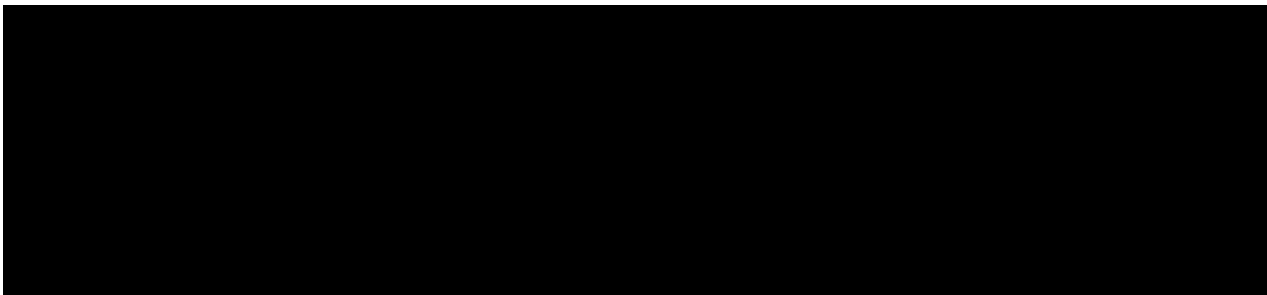


Table 2 Addition volume to prepare Primary culture medium for PMO-2

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	54.0 $\mu\text{L}$ of 925.1 $\mu\text{mol/L}^{*1}$	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	446.0 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 3 Addition volume to prepare Primary culture medium for PMO-3

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	60.7 $\mu\text{L}$ of 823.9 $\mu\text{mol/L}^{*1}$	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	439.3 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.



Table 4 Addition volume to prepare Primary culture medium for PMO-4

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	58.0 $\mu$ L of 861.4 $\mu$ mol/L <sup>*1</sup>	30.0 $\mu$ L of 100 $\mu$ mol/L	15.0 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500.0 $\mu$ L	442.0 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 5 Addition volume to prepare Primary culture medium for PMO-5

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	54.9 $\mu$ L of 910.2 $\mu$ mol/L <sup>*1</sup>	30.0 $\mu$ L of 100 $\mu$ mol/L	15.0 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500.0 $\mu$ L	445.1 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 6 Addition volume to prepare Primary culture medium for PMO-6

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	62.6 $\mu$ L of 798.1 $\mu$ mol/L <sup>*1</sup>	30.0 $\mu$ L of 100 $\mu$ mol/L	15.0 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500.0 $\mu$ L	437.4 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 7 Addition volume to prepare Primary culture medium for PMO-7

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	61.9 $\mu$ L of 807.9 $\mu$ mol/L <sup>*1</sup>	30.0 $\mu$ L of 100 $\mu$ mol/L	15.0 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500.0 $\mu$ L	438.1 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 8 Addition volume to prepare Primary culture medium for PMO-8

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	60.5 $\mu\text{L}$ of 826.3 $\mu\text{mol/L}$ * <sup>1</sup>	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	439.5 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 9 Addition volume to prepare Primary culture medium for PMO-9

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	64.2 $\mu\text{L}$ of 779.0 $\mu\text{mol/L}$ * <sup>1</sup>	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	435.8 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 10 Addition volume to prepare Primary culture medium for PMO-10

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	75.0 $\mu\text{L}$ of 667.0 $\mu\text{mol/L}$ * <sup>1</sup>	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	425.0 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 11 Addition volume to prepare Primary culture medium for PMO-11

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	132.5 $\mu\text{L}$ of 377.3 $\mu\text{mol/L}$ * <sup>1</sup>	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	367.5 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 12 Addition volume to prepare Primary culture medium for PMO-12

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	119.4 $\mu\text{L}$ of 418.9 $\mu\text{mol/L}$ *1	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	380.6 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 13 Addition volume to prepare Primary culture medium for PMO-13

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	54.8 $\mu\text{L}$ of 911.7 $\mu\text{mol/L}$ *1	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	445.2 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 14 Addition volume to prepare Primary culture medium for PMO-14

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	110.3 $\mu\text{L}$ of 453.5 $\mu\text{mol/L}$ *1	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	389.7 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 15 Addition volume to prepare Primary culture medium for PMO-15

	0 nmol/L	10 $\mu\text{mol/L}$ as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu\text{L}$	145.0 $\mu\text{L}$ of 344.9 $\mu\text{mol/L}$ *1	30.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$	15.0 $\mu\text{L}$ of 100 $\mu\text{mol/L}$
Primary culture medium	500.0 $\mu\text{L}$	355.0 $\mu\text{L}$	470.0 $\mu\text{L}$	485.0 $\mu\text{L}$

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 16 Addition volume to prepare Primary culture medium for PMO-16

	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	108.8 µL of 459.5 µmol/L* <sup>1</sup>	30.0 µL of 100 µmol/L	15.0 µL of 100 µmol/L
Primary culture medium	500.0 µL	391.2 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 17 Addition volume to prepare Primary culture medium for PMO-17

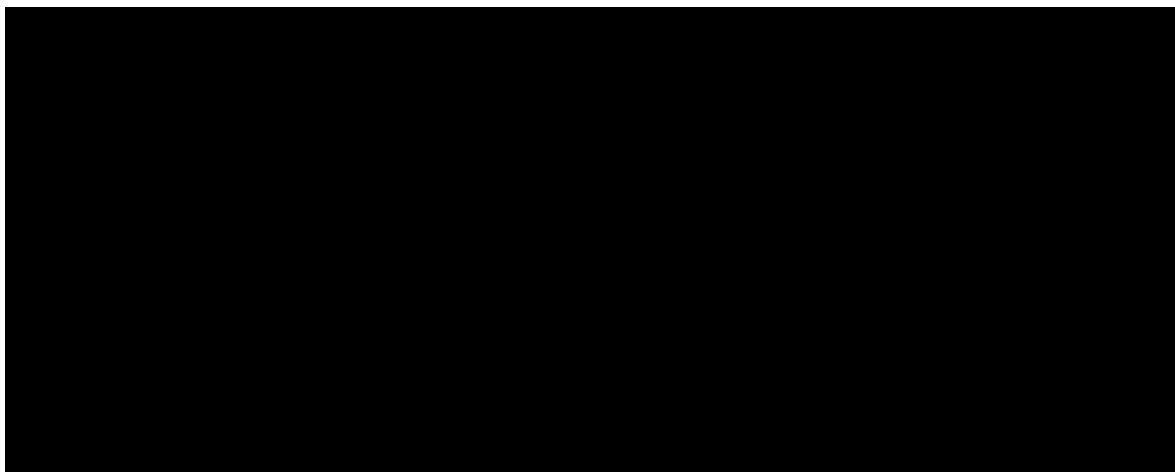
	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	121.8 µL of 410.6 µmol/L* <sup>1</sup>	30.0 µL of 100 µmol/L	15.0 µL of 100 µmol/L
Primary culture medium	500.0 µL	378.2 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

### 6.6.3 Preparation of Endo-Porter in Primary culture medium



### 6.6.4 Exposure



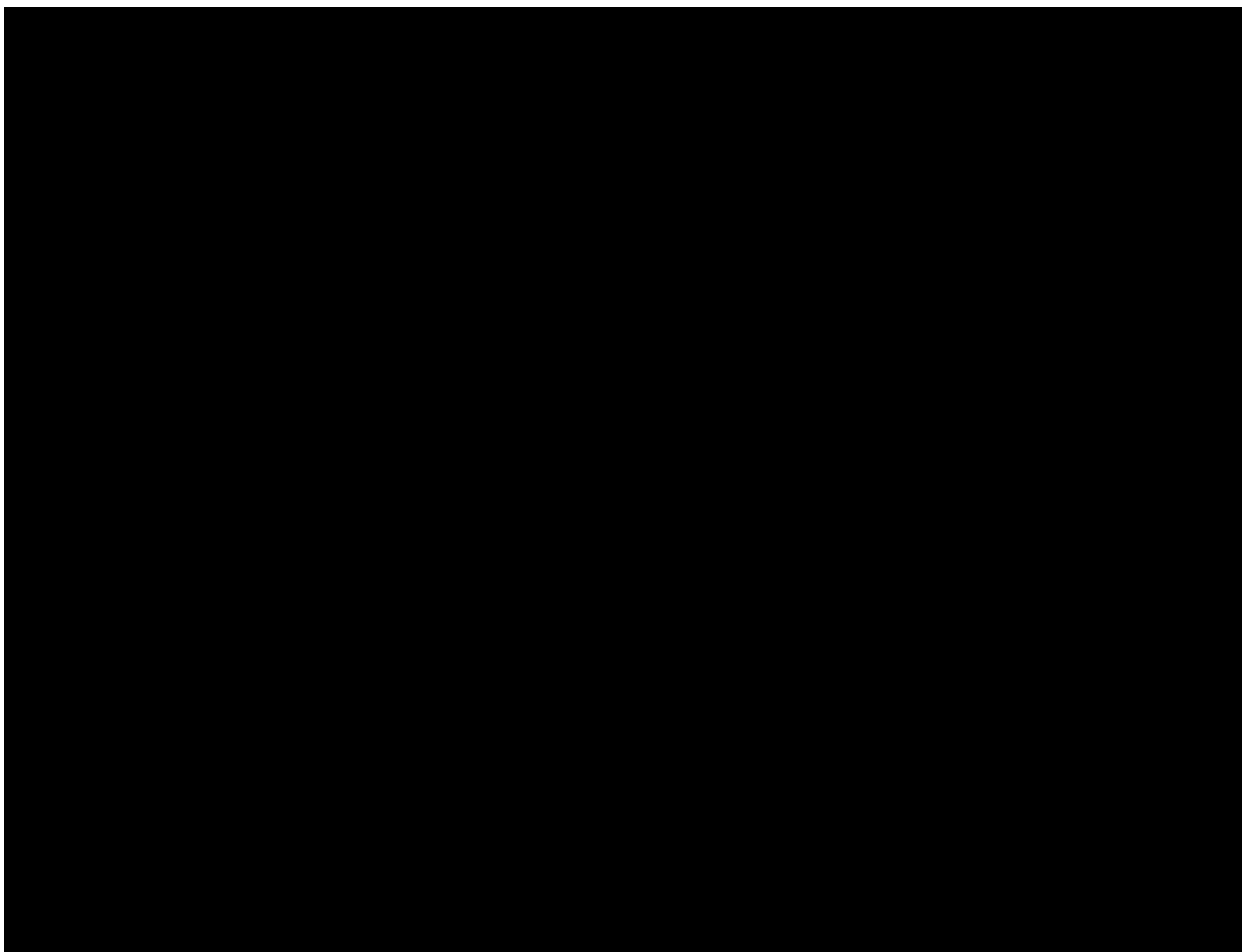
	1	2	3	4	5	6
A						
B						
C						
D						

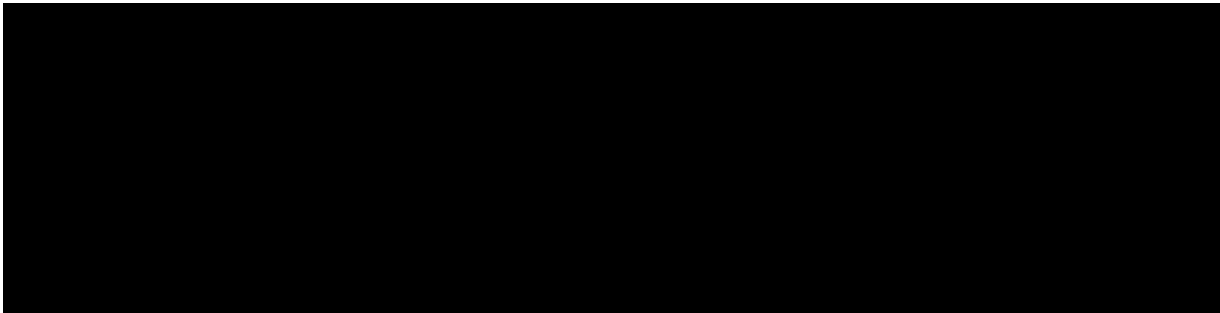
Fig. 1 Plate layout<sup>\*1</sup> of exposure to PMOs

\*1: Concentrations provided in the plate layout were as final concentration in each well.

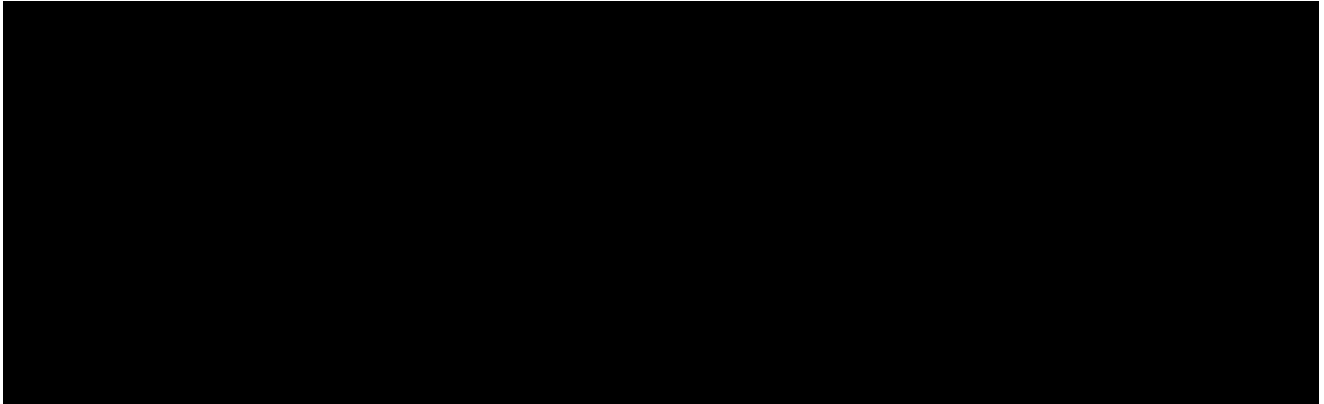
\*2: There was no cells in wells and only 500  $\mu$ L of fresh Primary culture medium without Endo-Porter DMSO was added at the time of transfection

#### 6.7 Gene Expression analysis





#### 6.7.4 Electrophoresis



Note: The 2<sup>nd</sup> run of each PMOs exon skipping activities was cancelled because it was requested from sponsor.

7. UNFORESEEABLE CIRCUMSTANCES THAT MIGHT HAVE AFFECTED TEST RELIABILITY AND DEVIATIONS FROM STUDY PLAN

[REDACTED]

8. RESULT

8.1 Concentration of PMO solutions

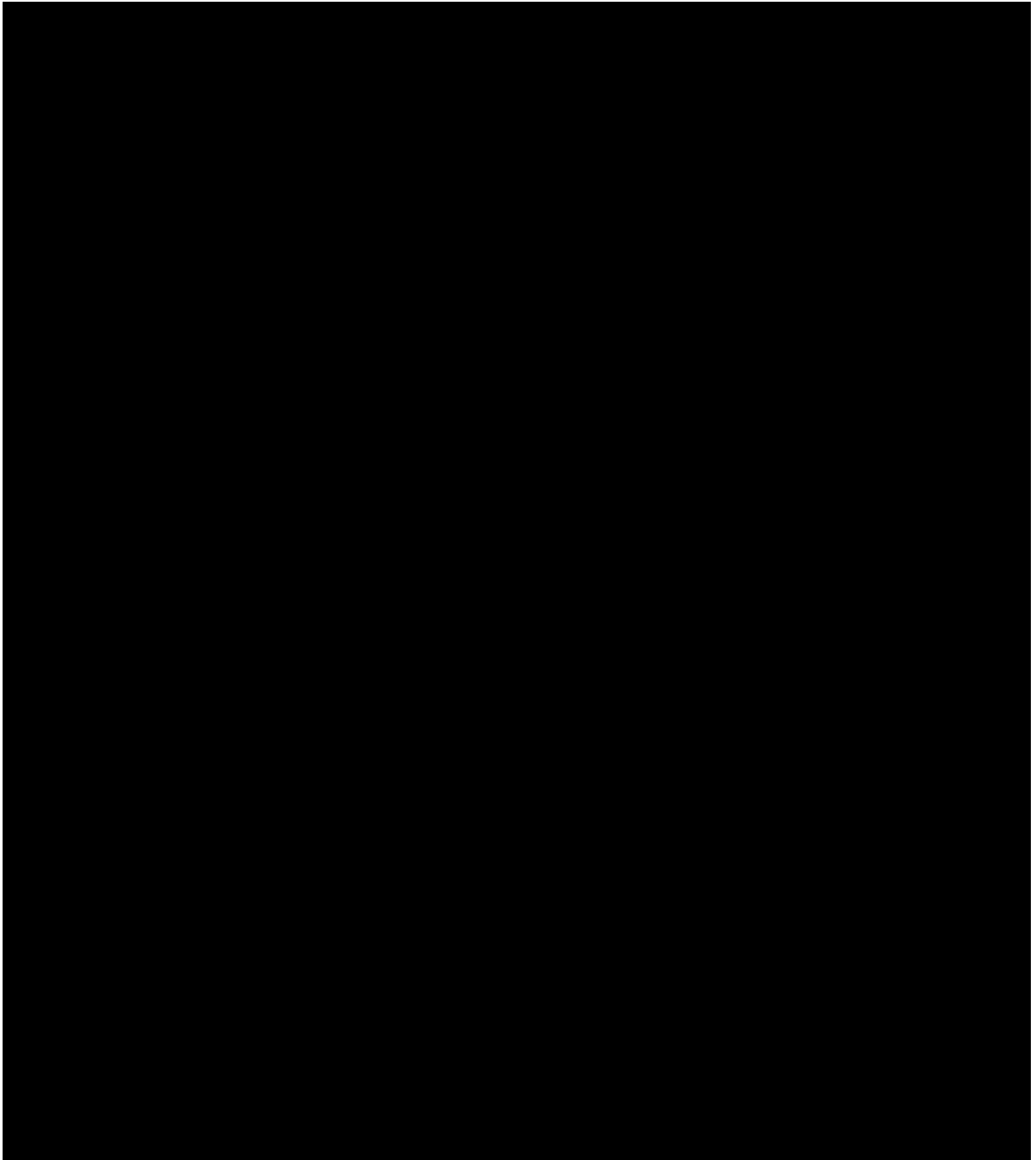


Table 18 Actual concentration of PMO solutions

A large solid black rectangular redaction box covering the entire content area of the table.



8.2 Assessment of skipping efficiency of PMOs



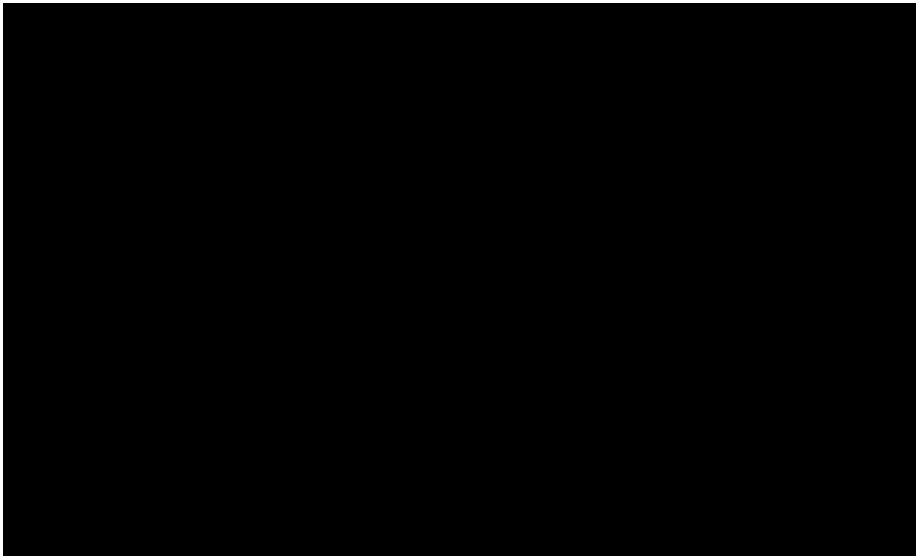


Fig. 2 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-2 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-2 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-2 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1: PMO-2 at 600 nmol/L (replicate 1)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

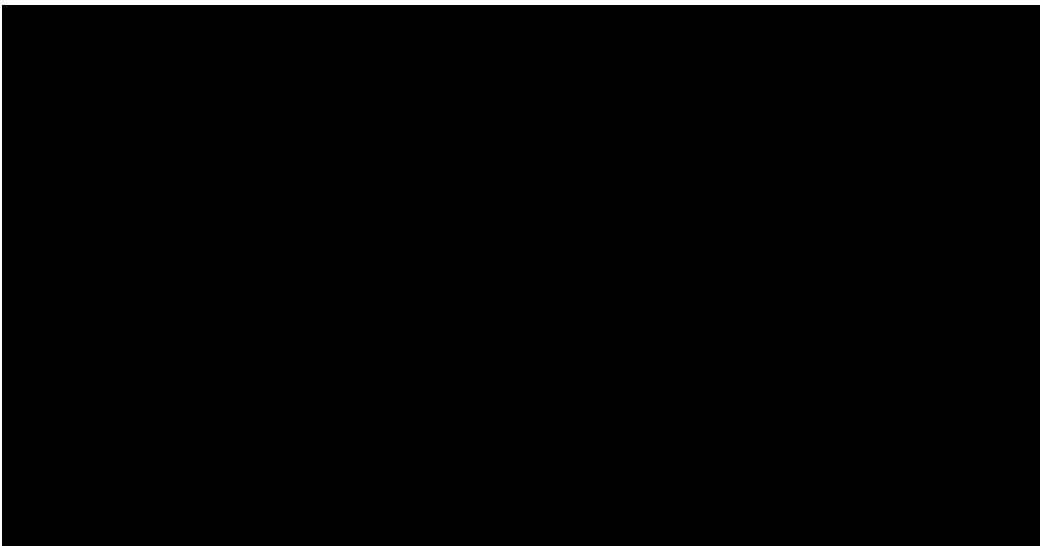


Fig. 3 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-2 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-2 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-2 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-2 at 600 nmol/L (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

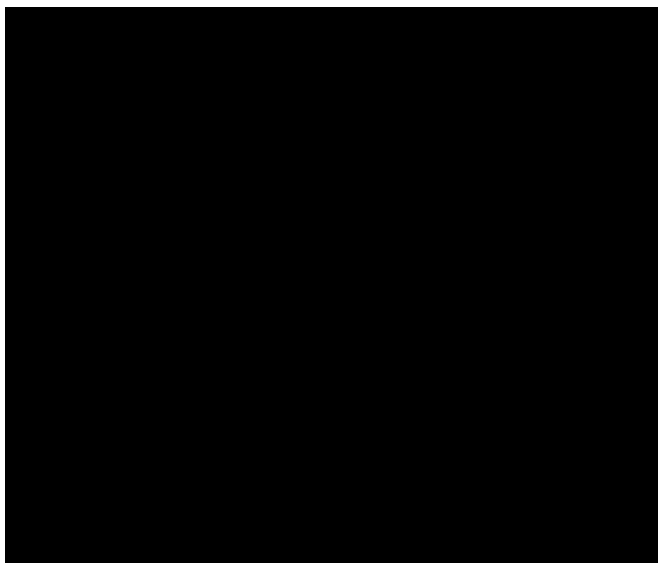


Fig. 4 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-2 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-2 at 10  $\mu\text{mol/L}$  (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

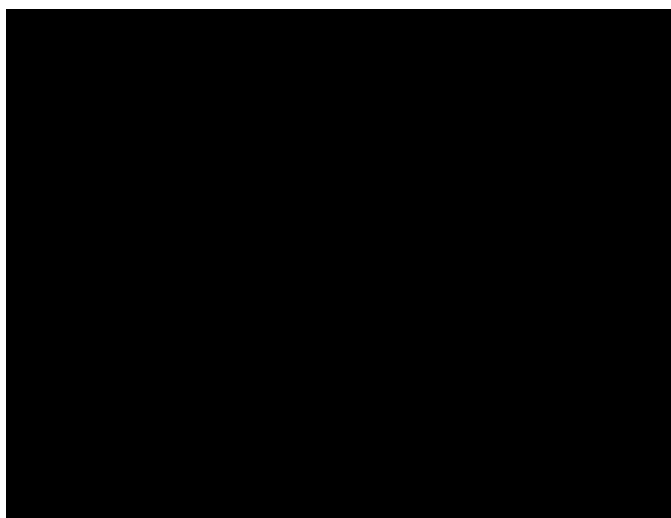


Fig. 5 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-3 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-3 (replicate 1, 2 and 3, respectively)

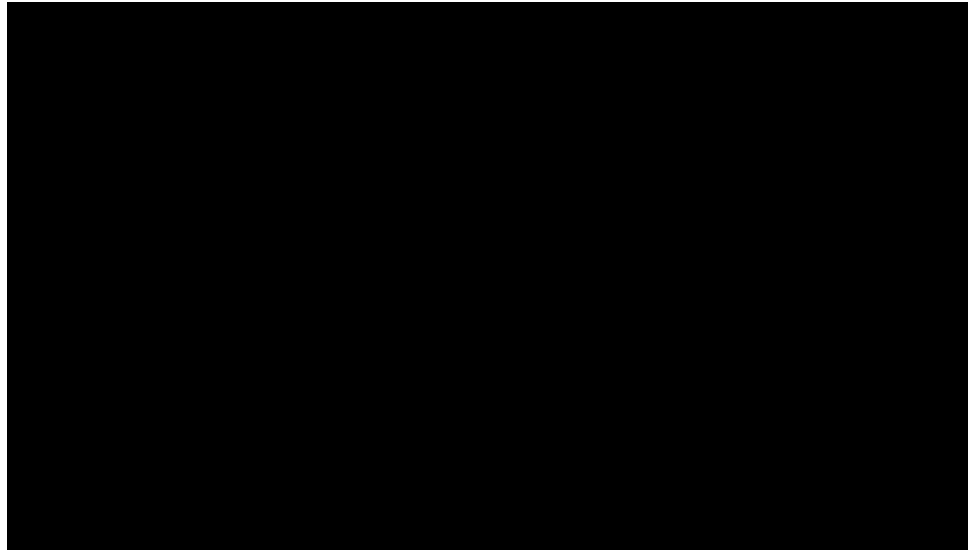


Fig. 6 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-3 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-3 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1 and F1: PMO-3 at 600 nmol/L (replicate 1 and 3, respectively), G1, H1 and A2: PMO-3 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

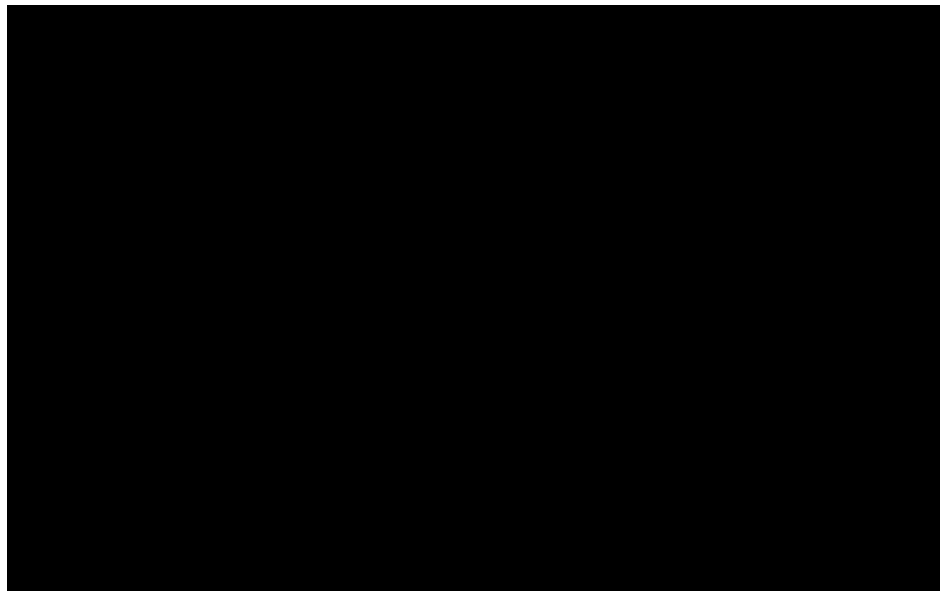


Fig. 7 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-3 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1: PMO-3 at 600 nmol/L (replicate 2)

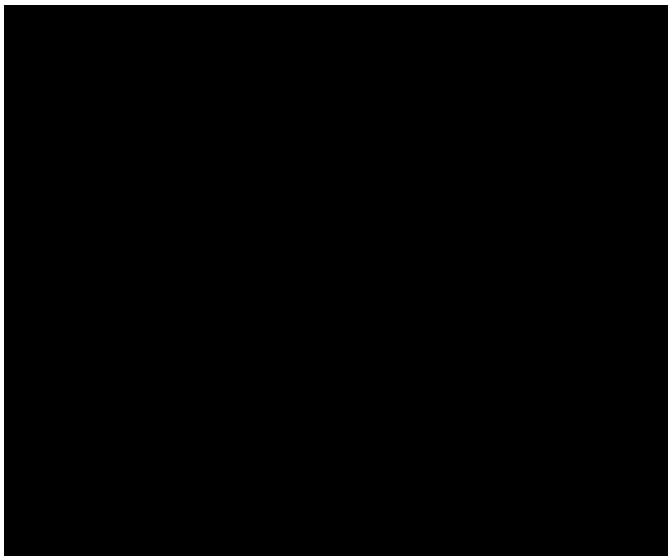


Fig. 8 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-3 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-3 (replicate 1, 2 and 3, respectively)

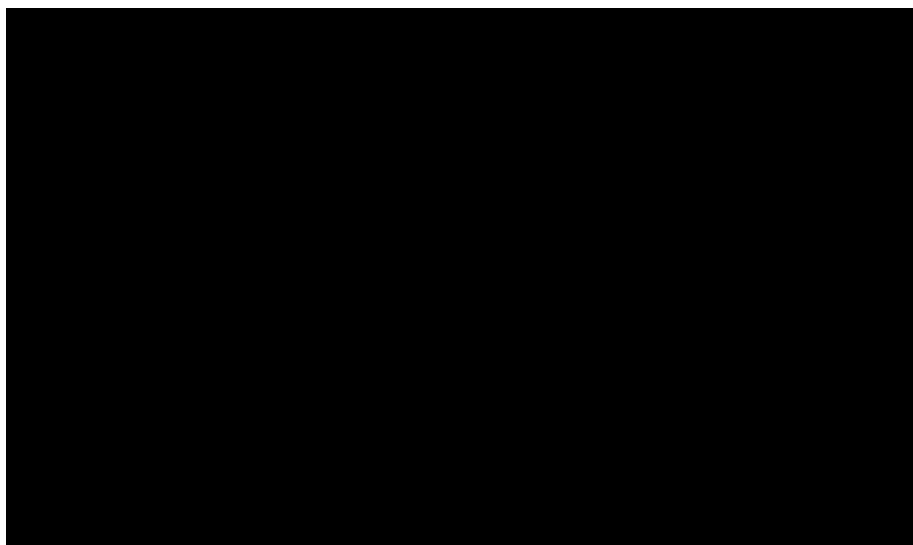


Fig. 9 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-3 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-3 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-3 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-3 at 10  $\mu$ mol/L (replicate 2 and 3, respectively)

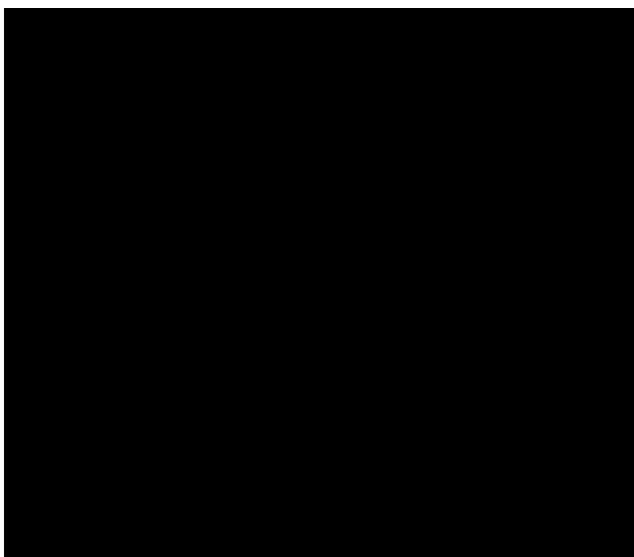


Fig. 10 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-3 exposed cells (Dilution factor 60, approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-3 at 10  $\mu$ mol/L (replicate 1)

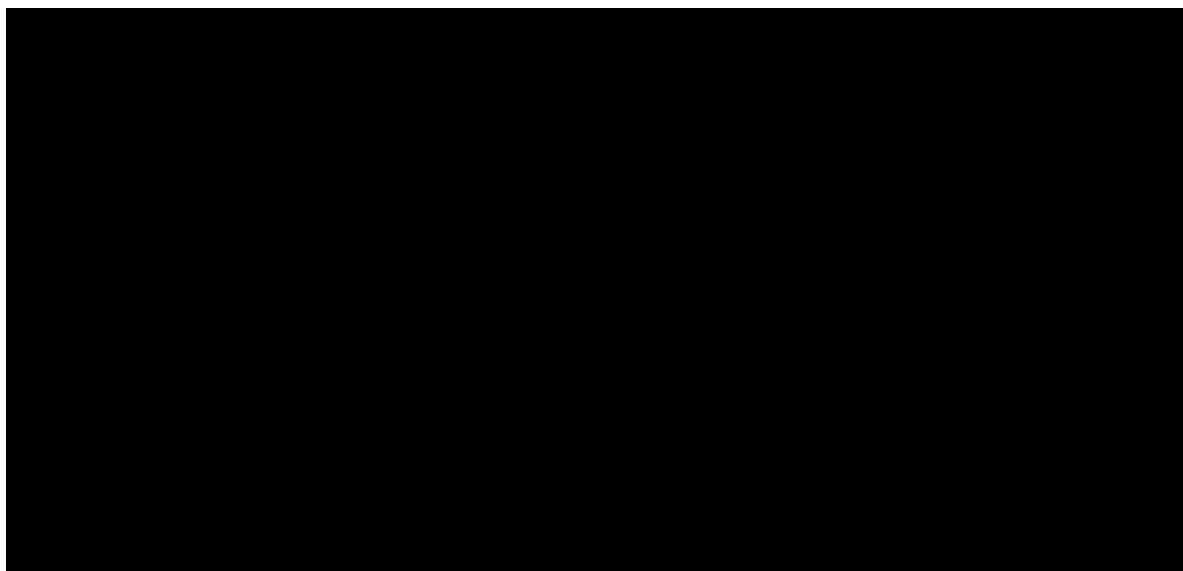


Fig. 11 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-4 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-4 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-4 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-4 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

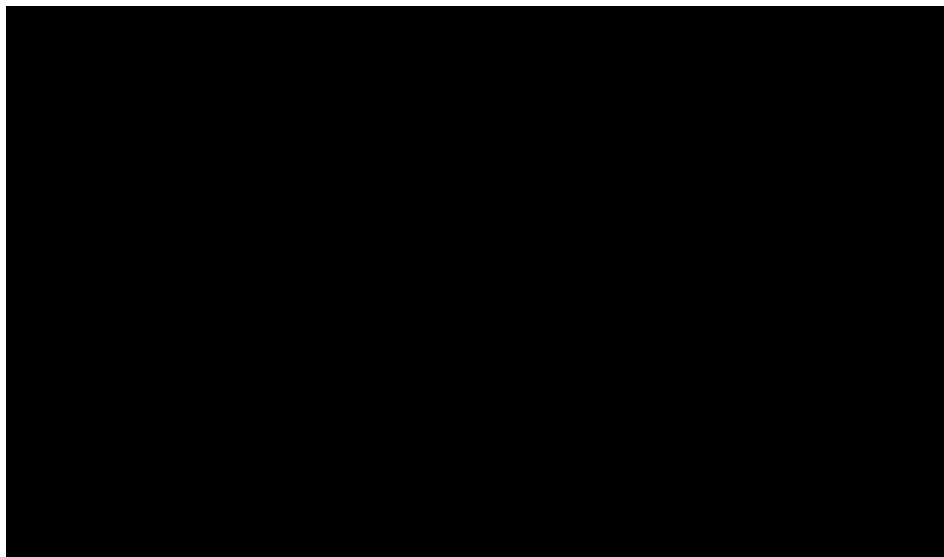


Fig. 12 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-4 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-4 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-4 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1: PMO-4 at 600 nmol/L (replicate 1)

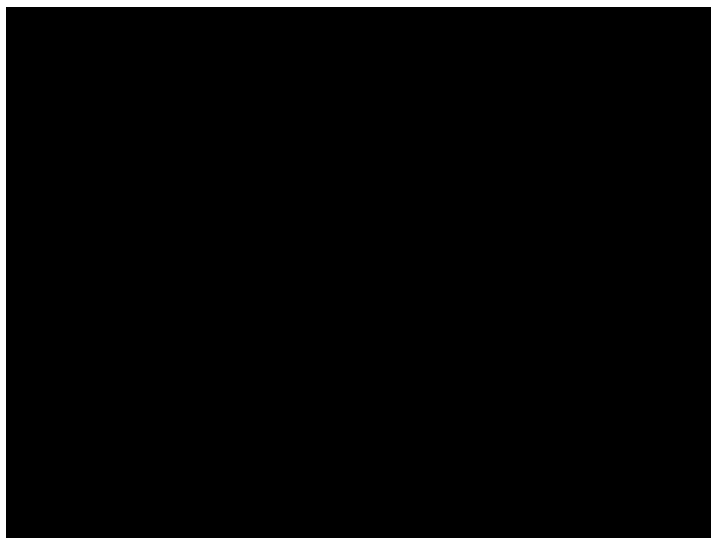


Fig. 13 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-4 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1 and C1: PMO-4 at 600 nmol/L (replicate 2 and 3, respectively), D1, E1 and F1: PMO-4 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

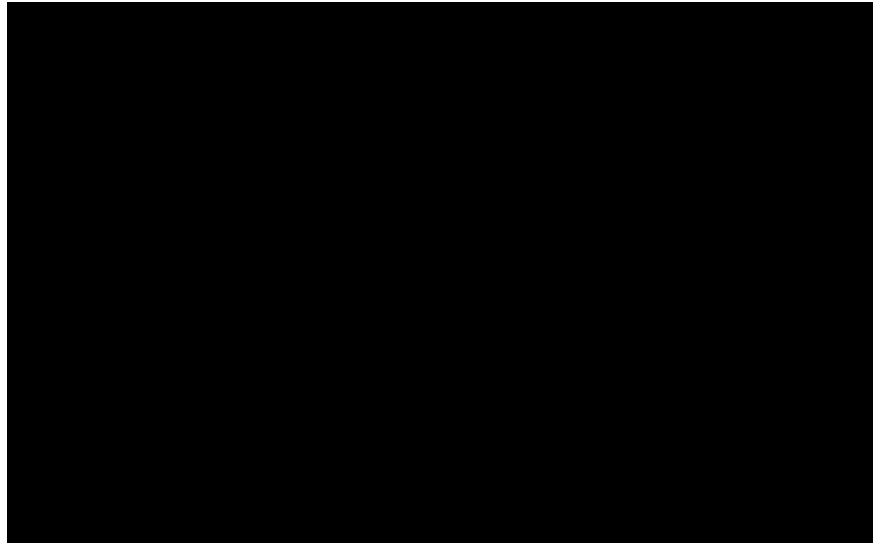


Fig. 14 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-5 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-5 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively)

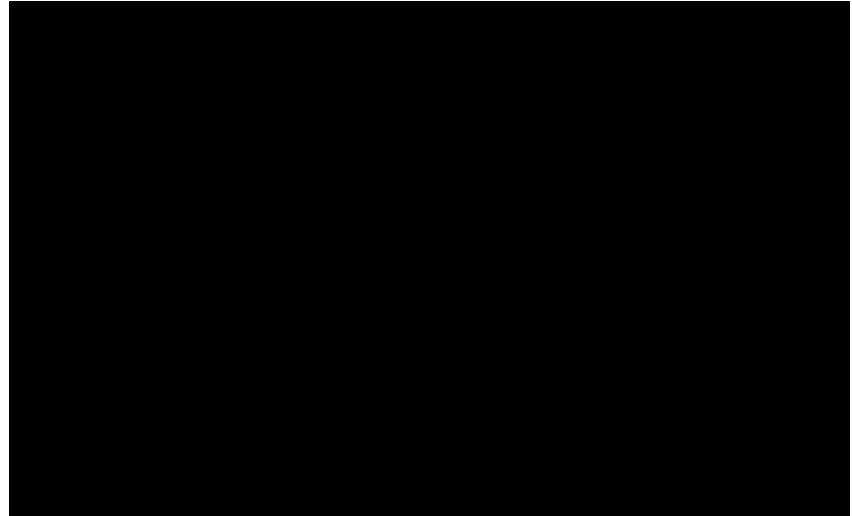


Fig. 15 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-5 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-5 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)



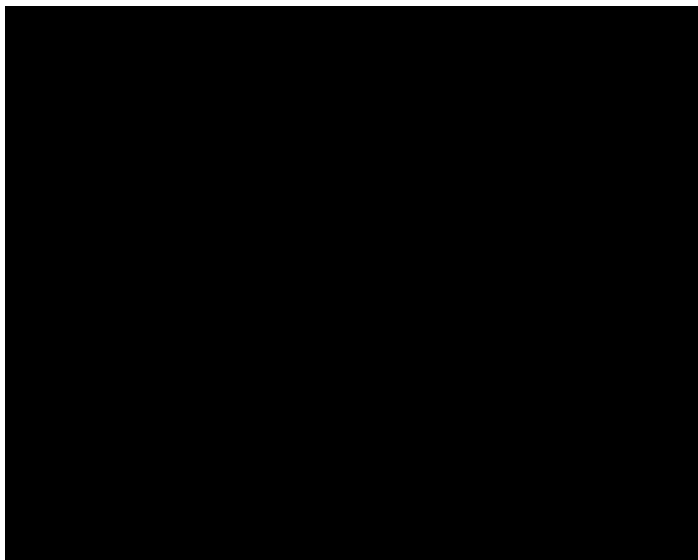


Fig. 16 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-5 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-5 (replicate 1, 2 and 3, respectively)

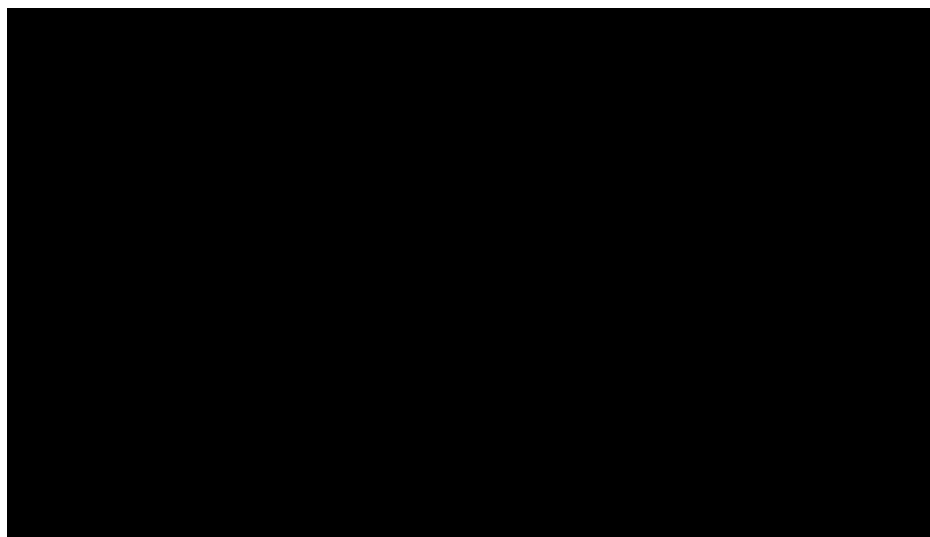


Fig. 17 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-5 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-5 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-5 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-5 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

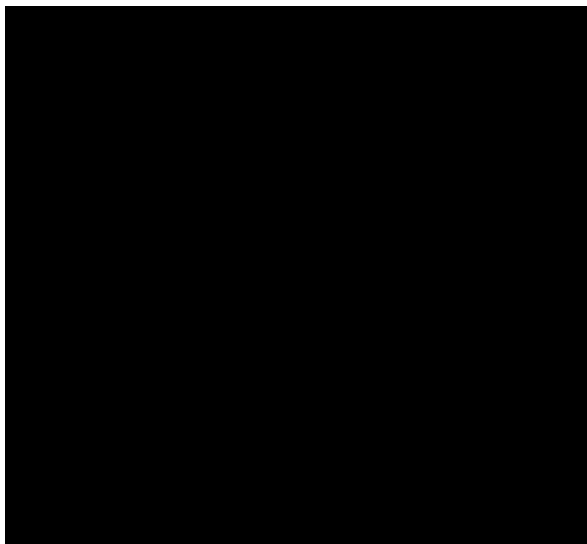


Fig. 18 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-6 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-6 (replicate 1, 2 and 3, respectively)

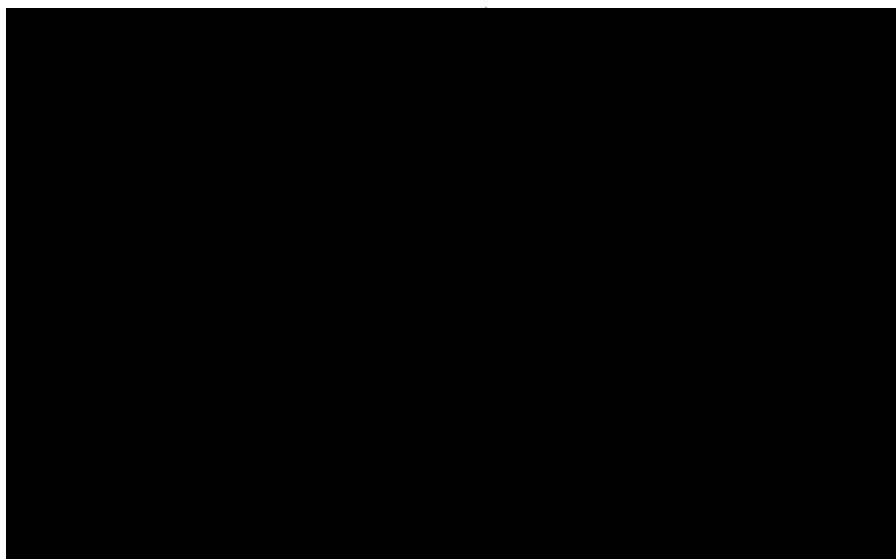


Fig. 19 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-6 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-6 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-6 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-6 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

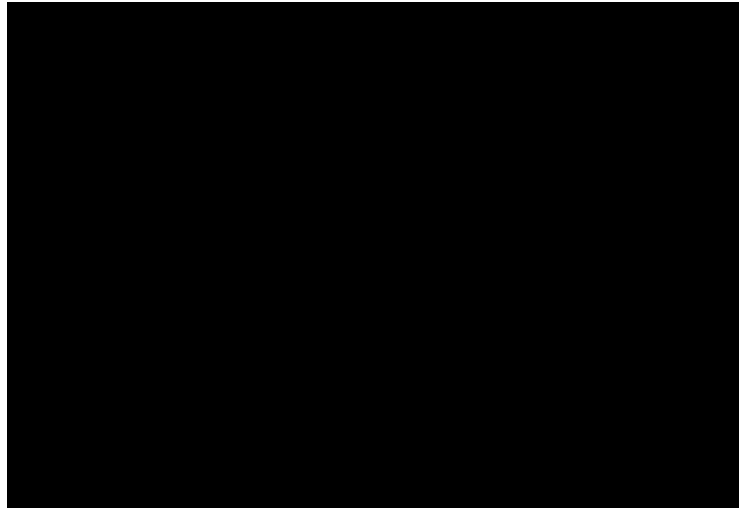


Fig. 20 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-6 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-6 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-6 at 300 nmol/L (replicate 1, 2 and 3, respectively)

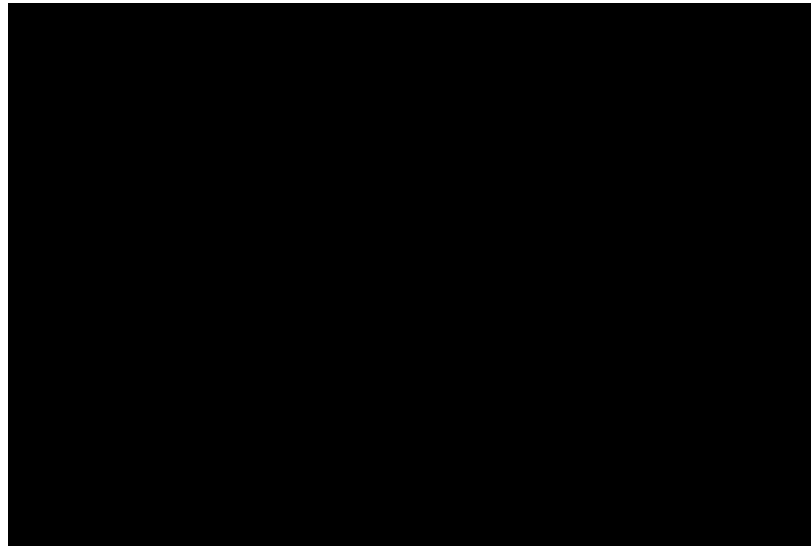


Fig. 21 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-6 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-6 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-6 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

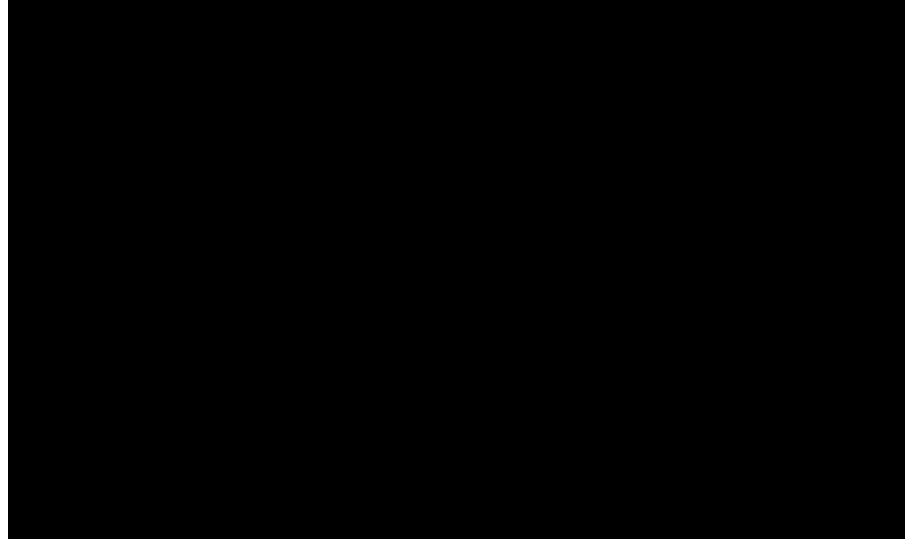


Fig. 22 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-7 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-7 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-7 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-7 at 600 nmol/L (replicate 1 and 2, respectively)

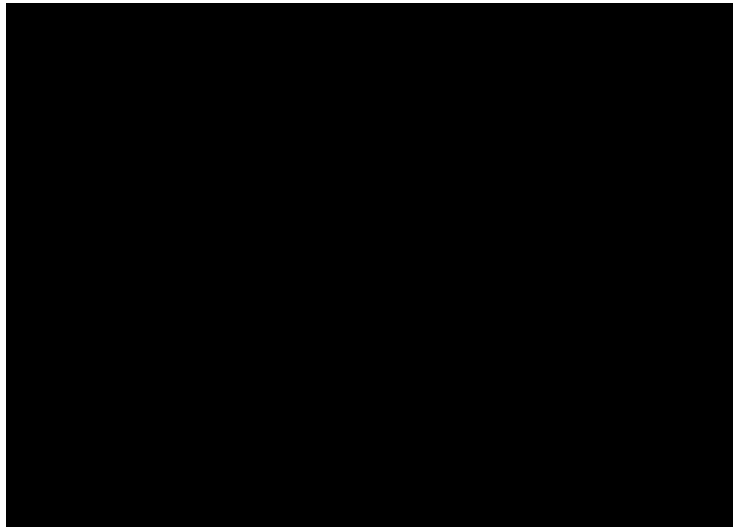


Fig. 23 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-7 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1: PMO-7 at 600 nmol/L (replicate 3), C1, D1 and E1: PMO-7 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

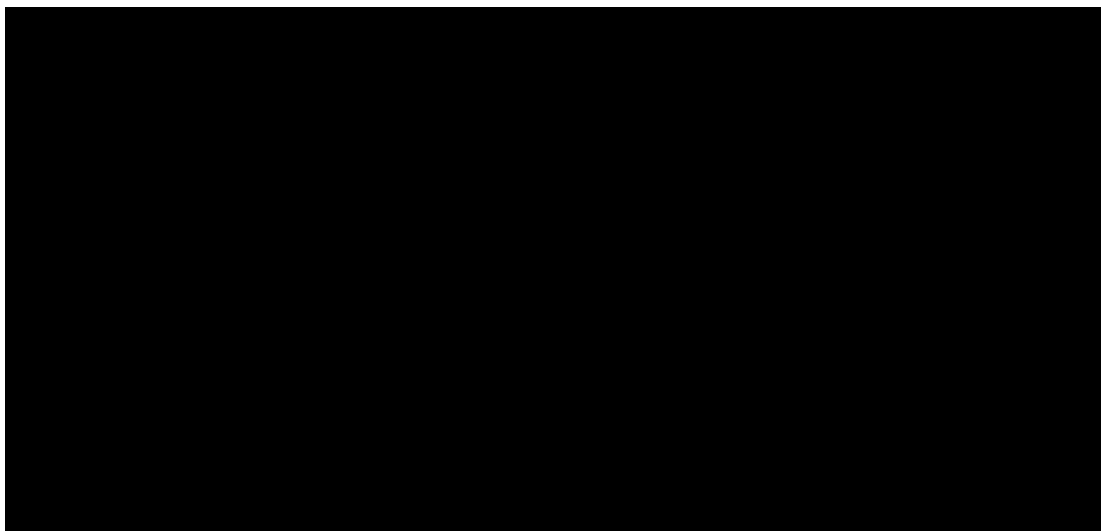


Fig. 24 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-7 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-7 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-7 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-7 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-7 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

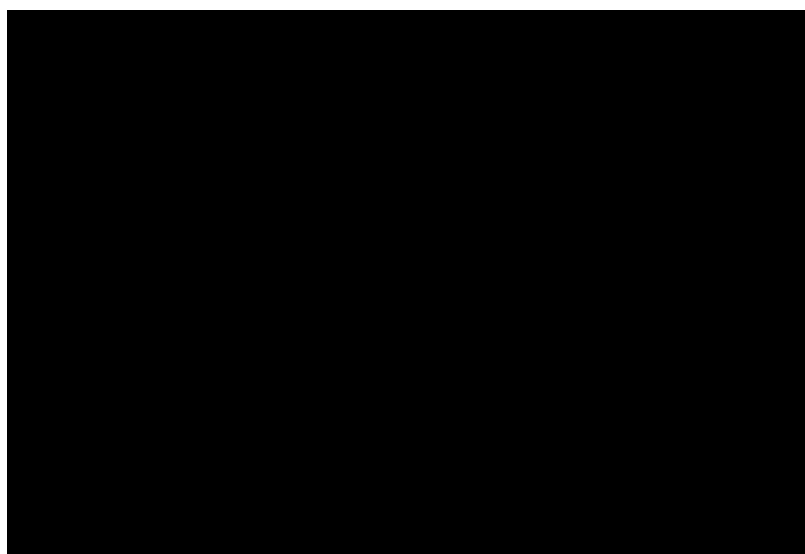


Fig. 25 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-8 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-8 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-8 at 300 nmol/L (replicate 1, 2 and 3, respectively)

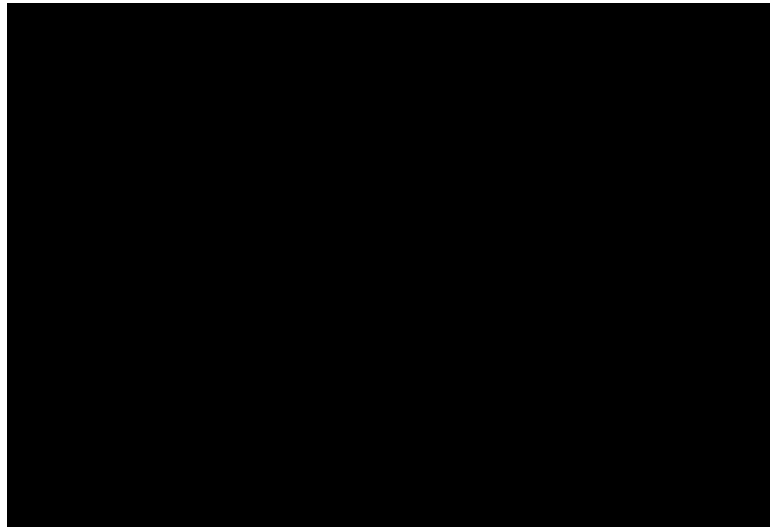


Fig. 26 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-8 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-8 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-8 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

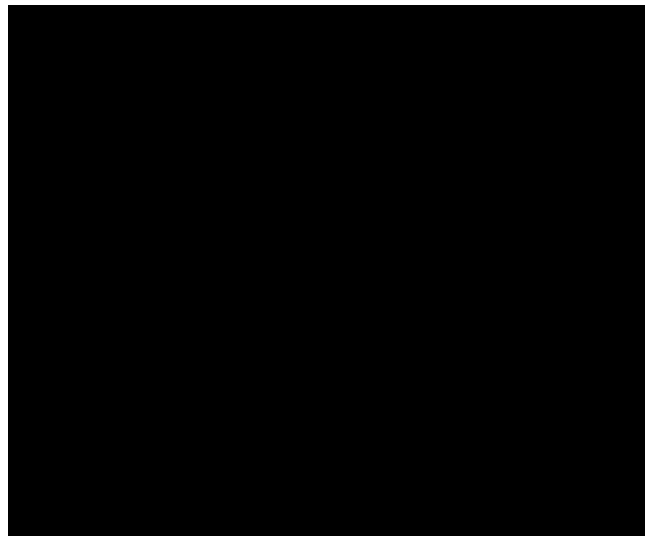


Fig. 27 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-8 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-8 (replicate 1, 2 and 3, respectively)

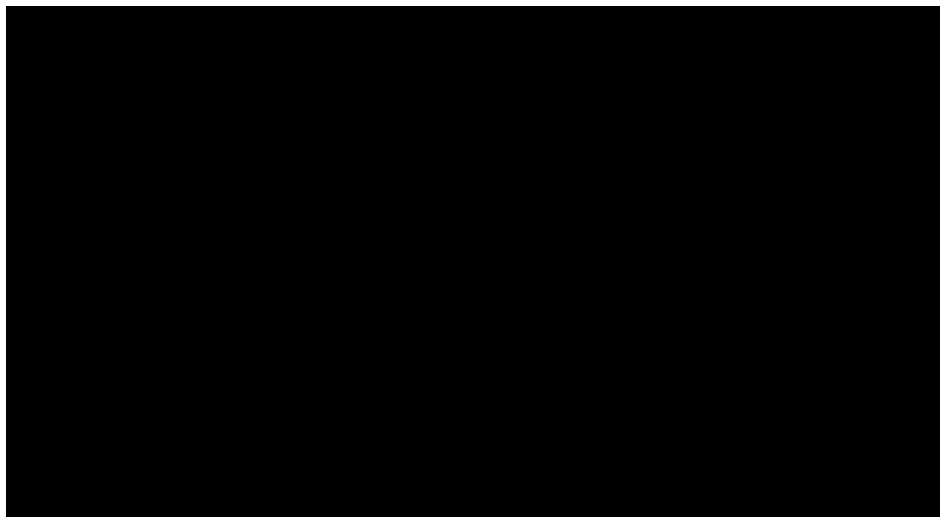


Fig. 28 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-8 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-8 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-8 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-8 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

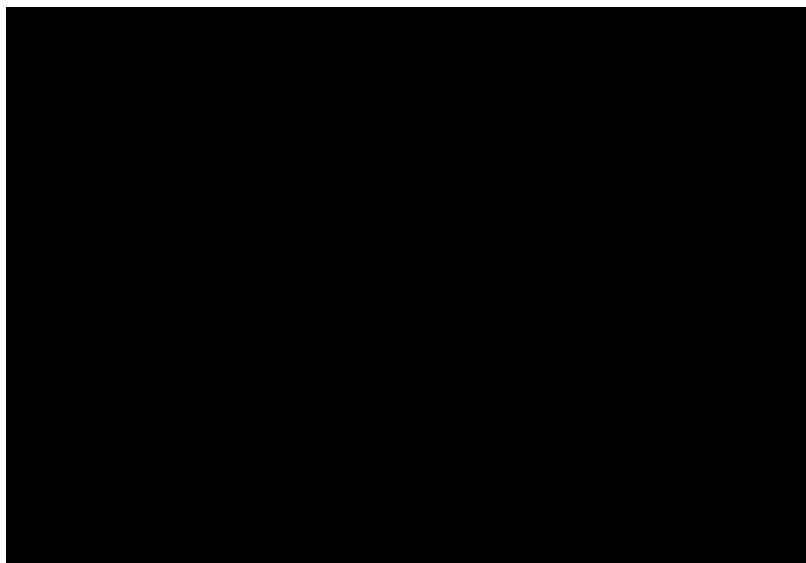


Fig. 29 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-9 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-9 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-9 at 300 nmol/L (replicate 1, 2 and 3, respectively)

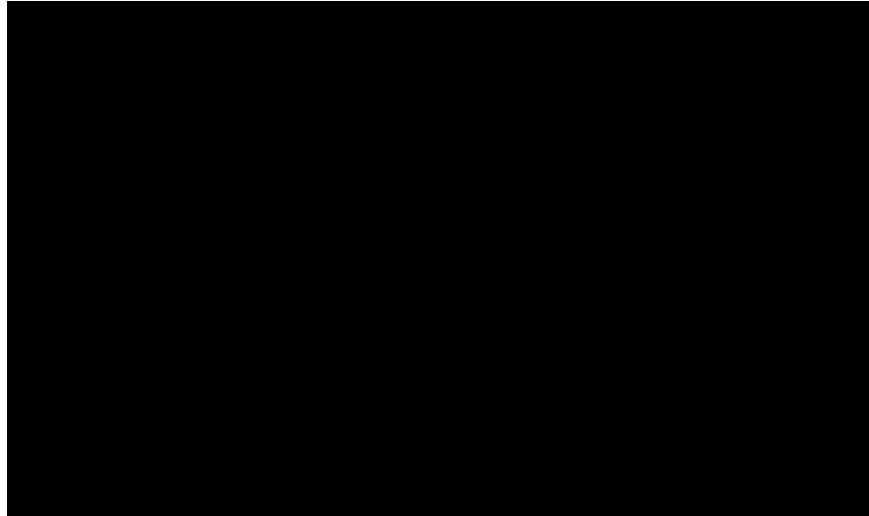


Fig. 30 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-9 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-9 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-9 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-9 at 600 nmol/L (replicate 1 and 2, respectively)

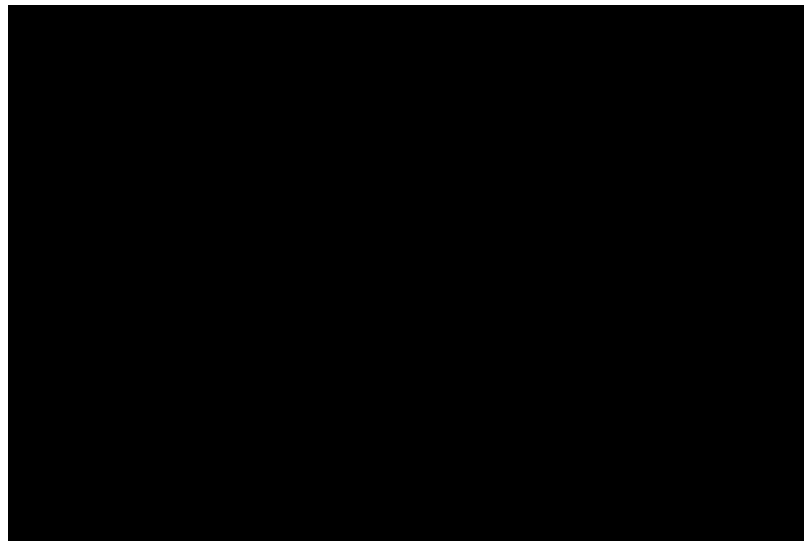


Fig. 31 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-9 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1: PMO-9 at 600 nmol/L (replicate 3), C1, D1 and E1: PMO-9 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.



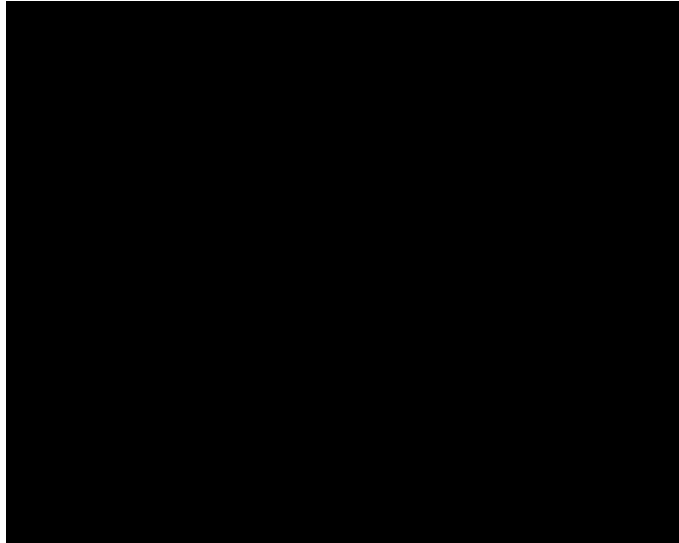


Fig. 32 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-10 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-10 (replicate 1, 2 and 3, respectively)

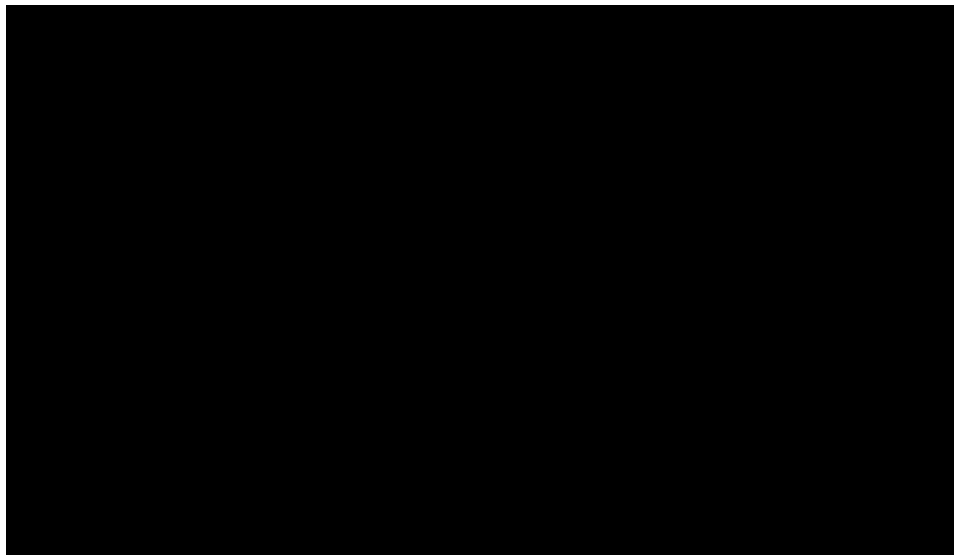


Fig. 33 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-10 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-10 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-10 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-10 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

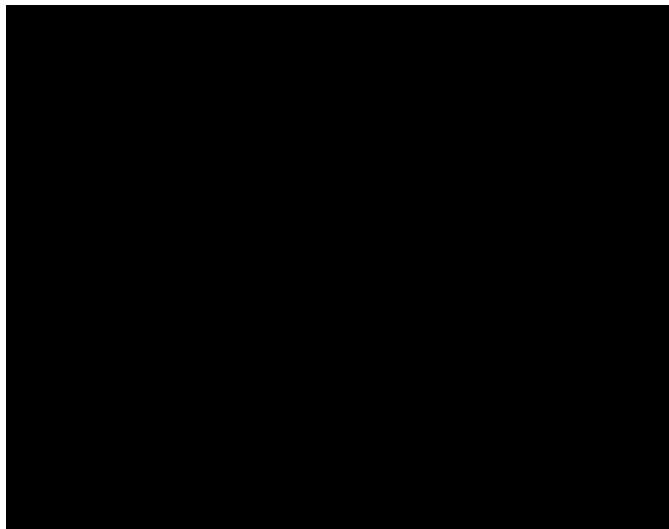


Fig. 34 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-10 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-10 (replicate 1, 2 and 3, respectively)

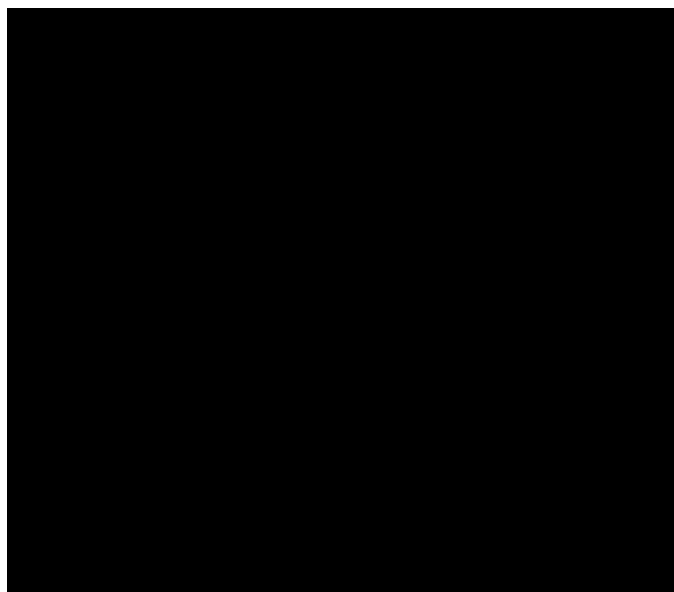


Fig. 35 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-10 exposed cells (Dilution factor 60, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-10 at 300 nmol/L (replicate 1, 2 and 3, respectively)



Fig. 36 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-10 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-10 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-10 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

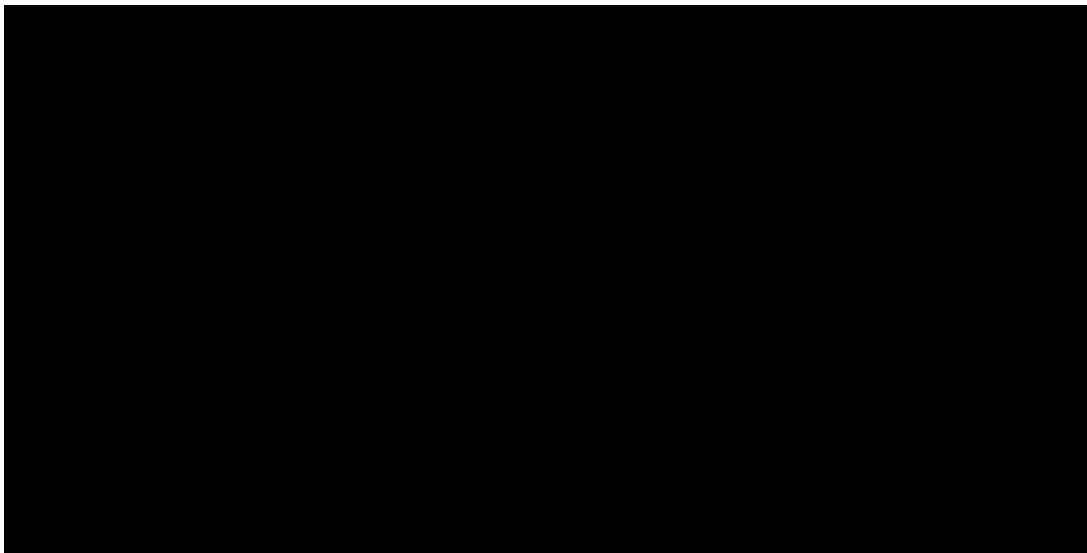


Fig. 37 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-11 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-11 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-11 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-11 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-11 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

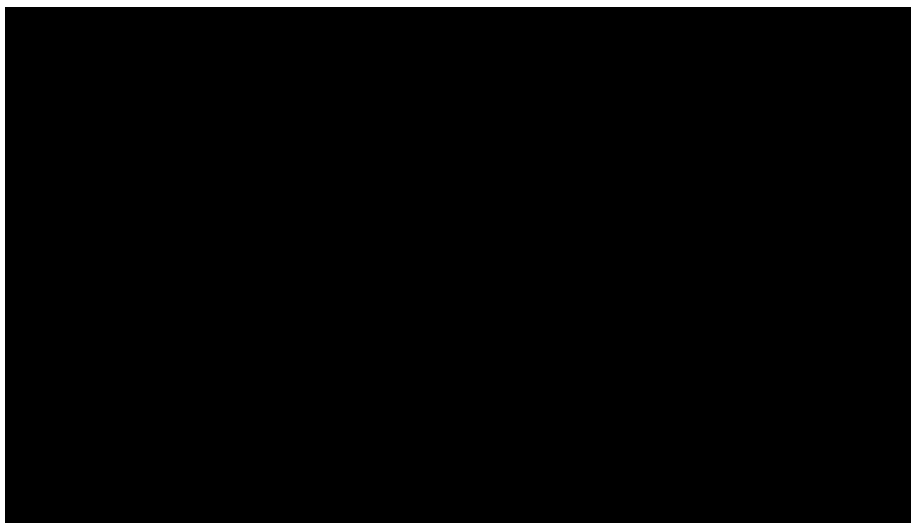


Fig. 38 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-11 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-11 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-11 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-11 at 600 nmol/L (replicate 1 and 2, respectively)

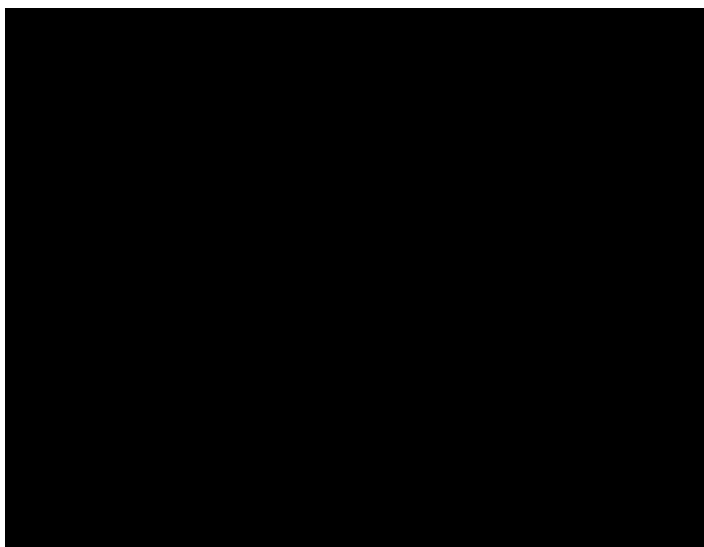


Fig. 39 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-11 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-11 at 600 nmol/L (replicate 3), C1, D1 and E1: PMO-11 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

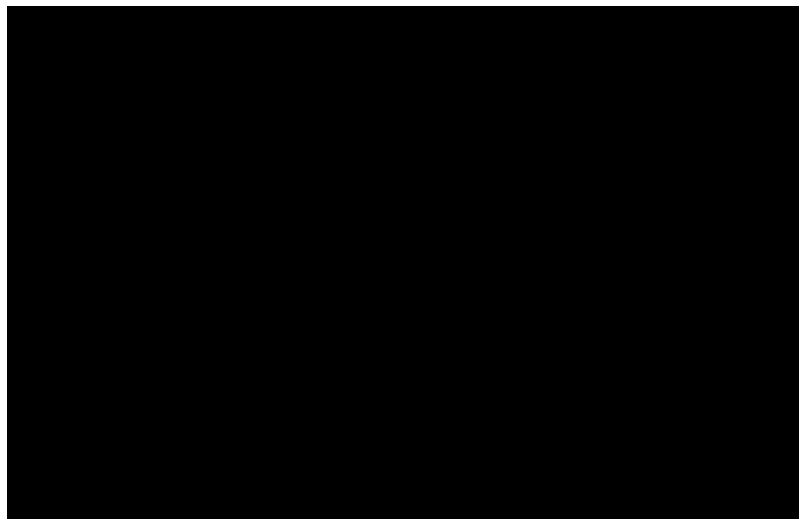


Fig. 40 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-12 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-12 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-12 at 300 nmol/L (replicate 1, 2 and 3, respectively)

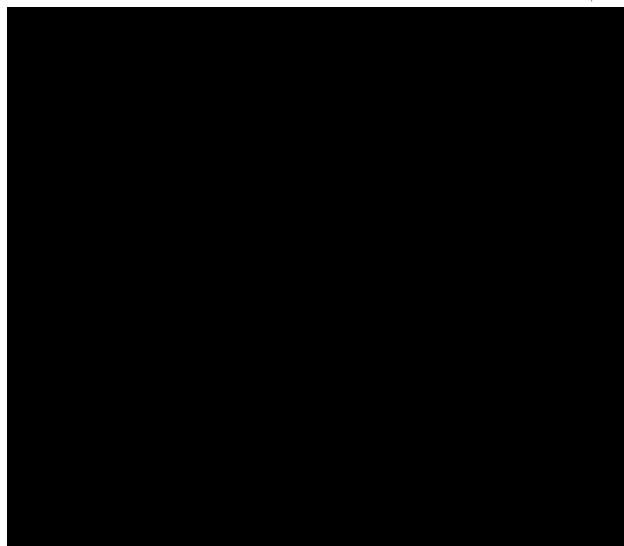


Fig. 41 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-12 exposed cells (Dilution factor 40, approximately 153 bp fragment)

A1: Ladder Marker, B1: PMO-12 at 600 nmol/L (replicate 1)

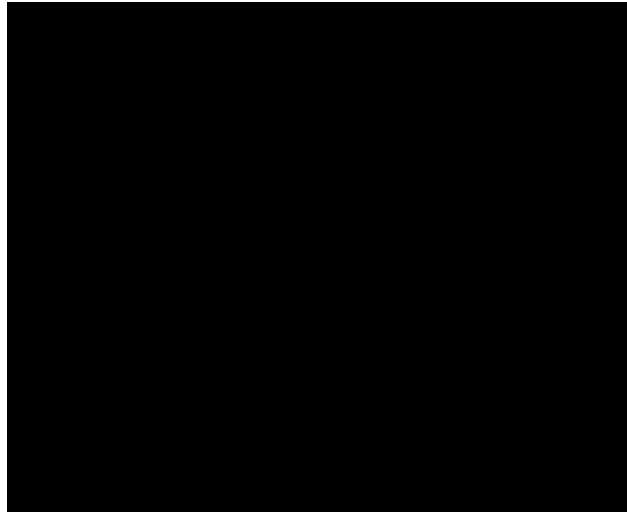


Fig. 42 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-12 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1 and C1: PMO-12 at 600 nmol/L (replicate 2 and 3, respectively)

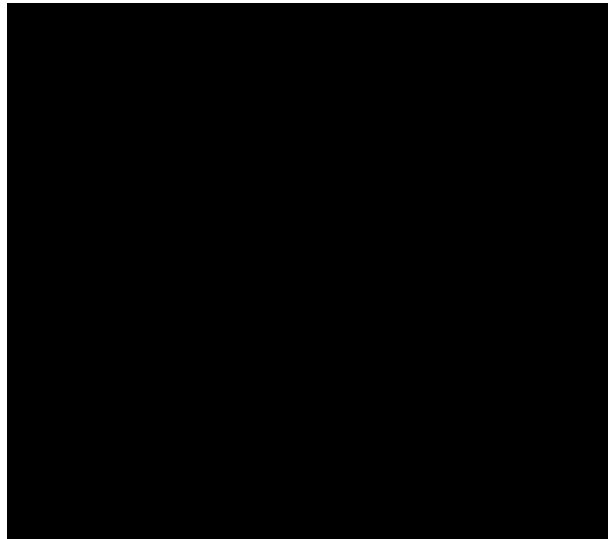


Fig. 43 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-12 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-12 (replicate 1, 2 and 3, respectively)

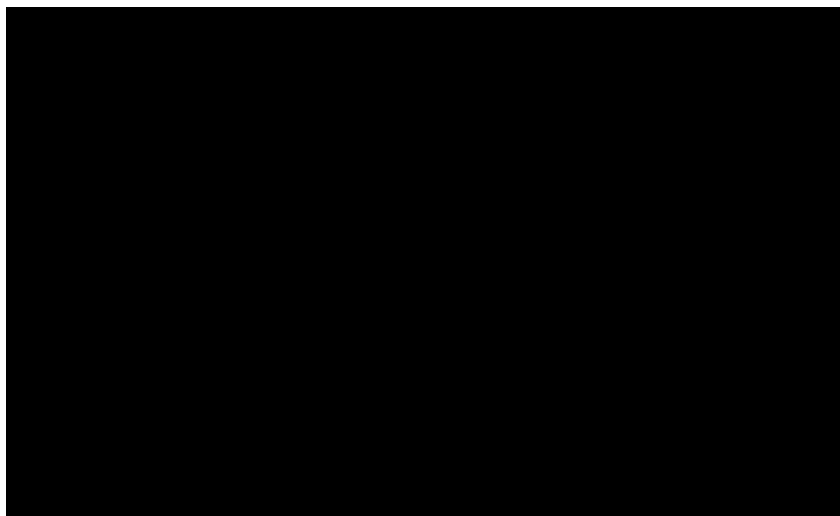


Fig. 44 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-12 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-12 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1 and F1: PMO-12 at 600 nmol/L (replicate 2 and 3, respectively), G1, H1 and A2: PMO-12 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

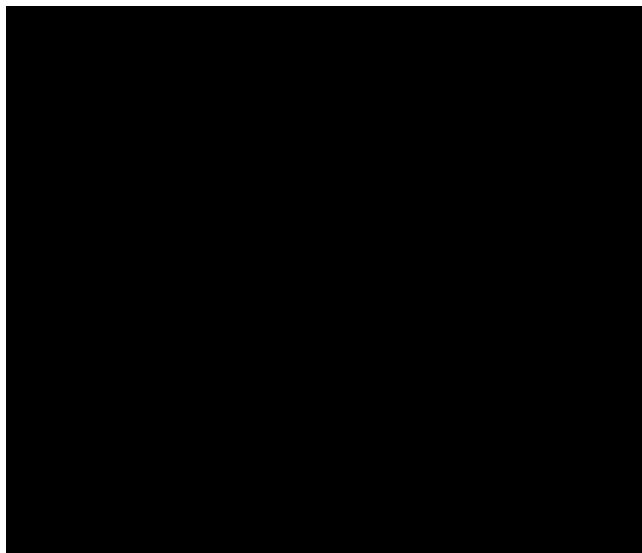


Fig. 45 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-12 exposed cells (Dilution factor 60 approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-12 at 600 nmol/L (replicate 1)

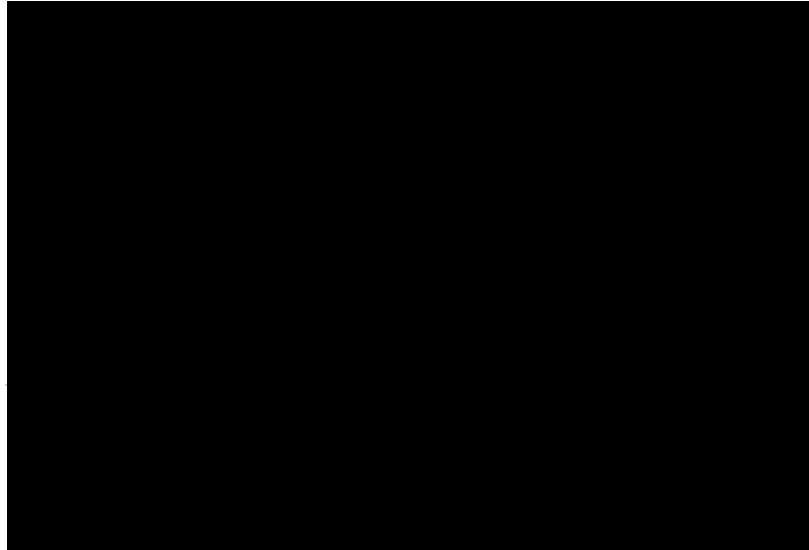


Fig. 46 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-13 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-13 (replicate 1, 2 and 3, respectively), E1 and F1: PMO-13 at 300 nmol/L (replicate 1 and 3, respectively)

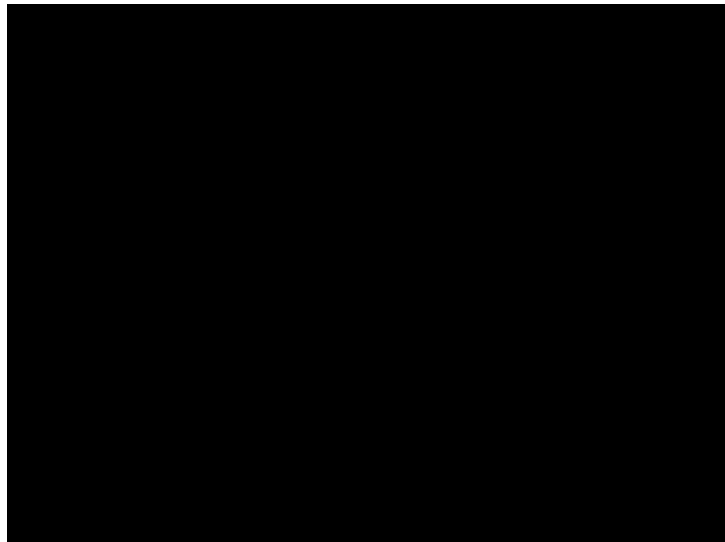


Fig. 47 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-13 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1: PMO-13 at 300 nmol/L (replicate 2)



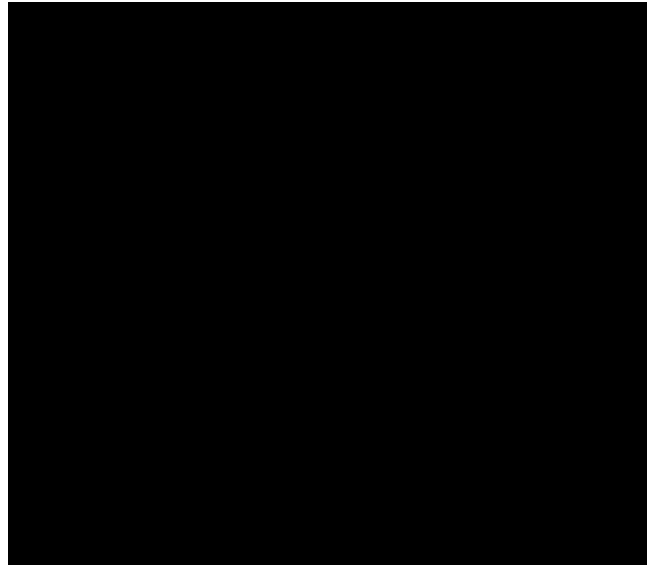


Fig. 48 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-13 exposed cells (Dilution factor 40, approximately 153 bp fragment)  
A1: Ladder Marker, B1: PMO-13 at 600 nmol/L (replicate 1)

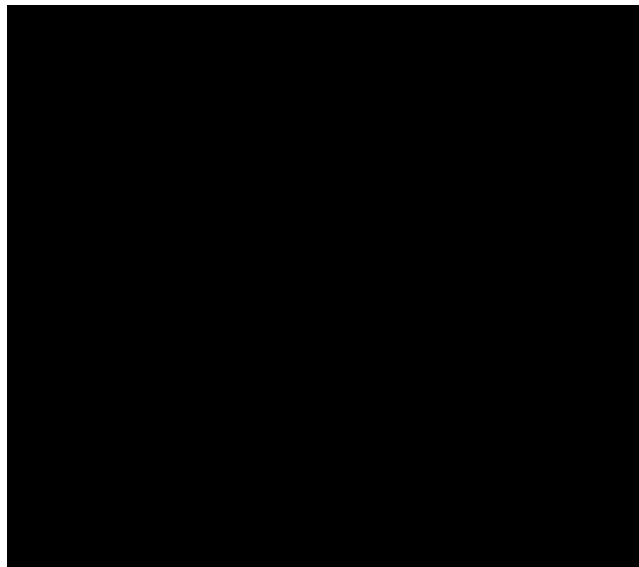


Fig. 49 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-13 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1: PMO-13 at 600 nmol/L (replicate 3)

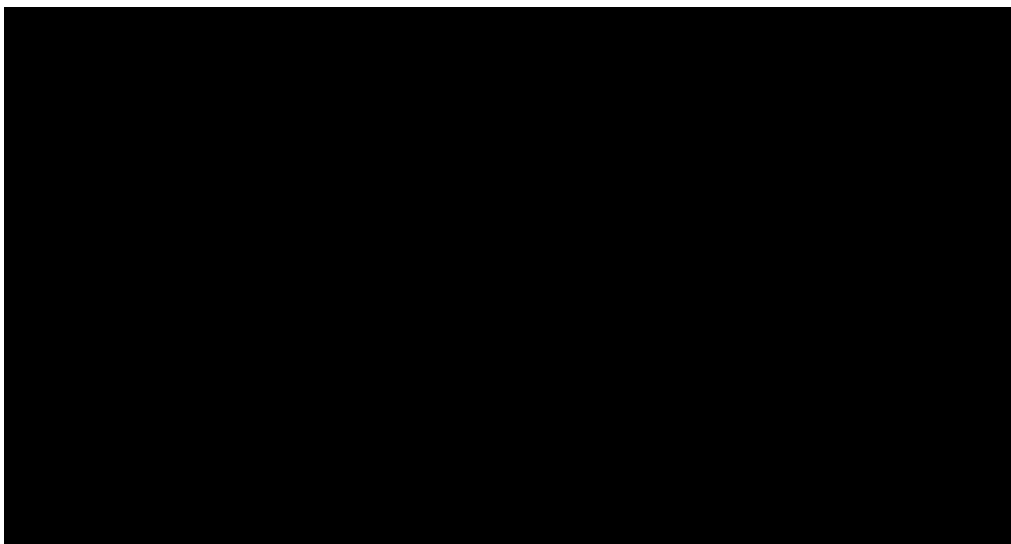


Fig. 50 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-13 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-13 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-13 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1: PMO-13 at 600 nmol/L (replicate 3), A2, B2 and C2: PMO-13 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

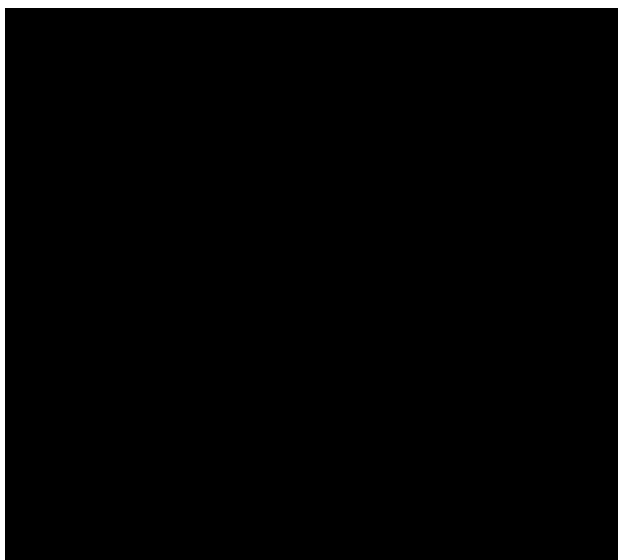


Fig. 51 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-13 exposed cells (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-13 at 600 nmol/L (replicate 1)

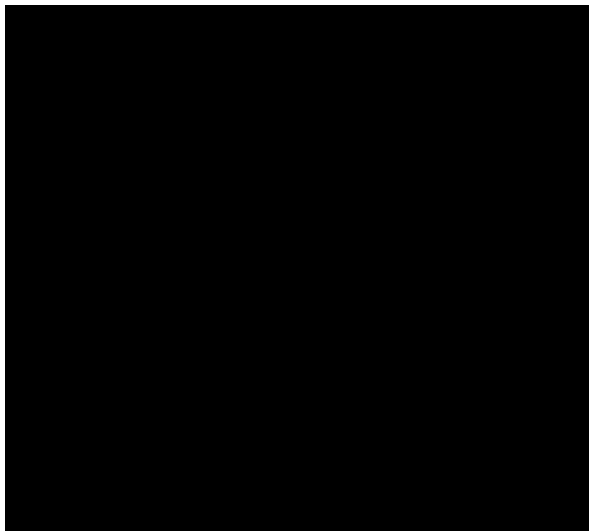


Fig. 52 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-13 exposed cells (Dilution factor 100, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1: PMO-13 at 600 nmol/L (replicate 2)

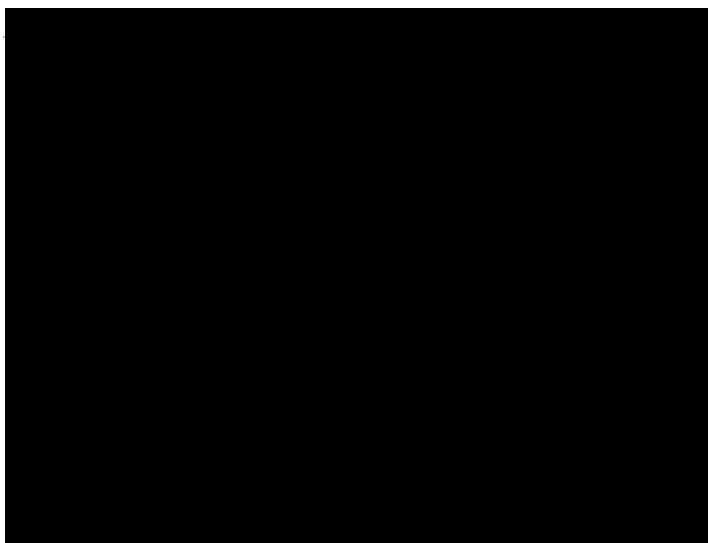


Fig. 53 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-14 (replicate 1, 2 and 3, respectively)

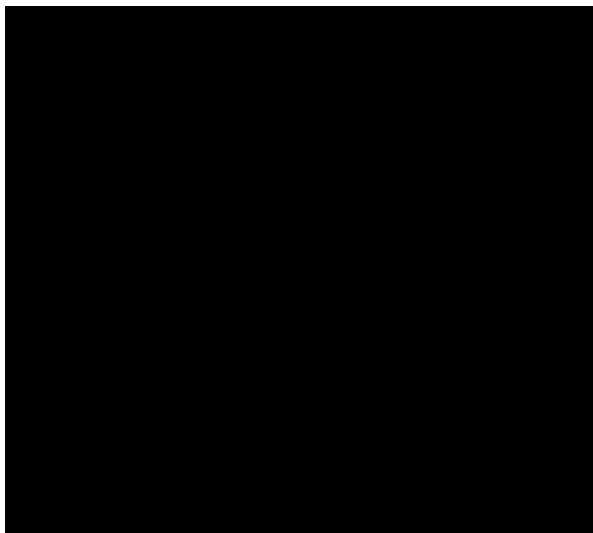


Fig. 54 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1 and C1: PMO-14 at 300 nmol/L (replicate 1 and 2, respectively)

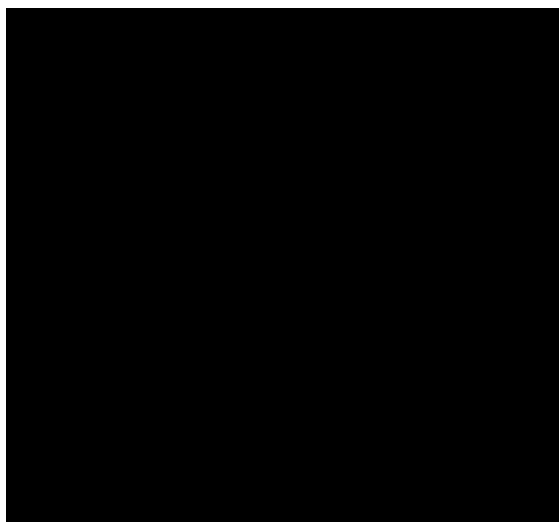


Fig. 55 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 40, approximately 153 bp fragment)  
A1: Ladder Marker, B1: PMO-14 at 300 nmol/L (replicate 3)

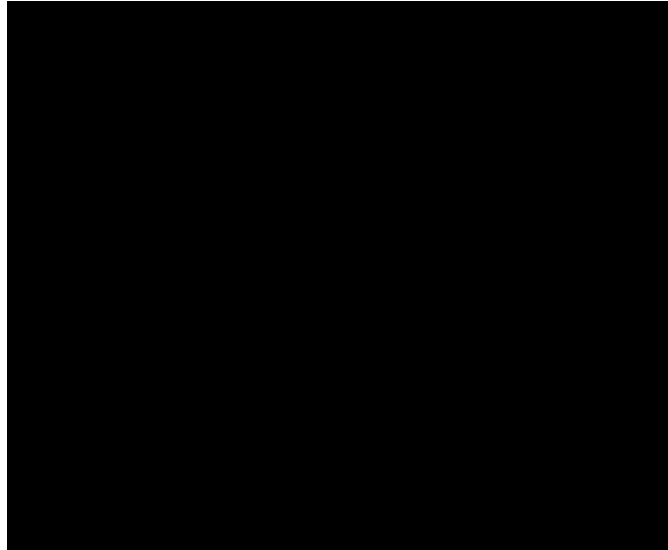


Fig. 56 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1: control of PMO-14 (replicate 3), C1 and D1: PMO1-14 at 300 nmol/L (replicate 1 and 2, respectively)

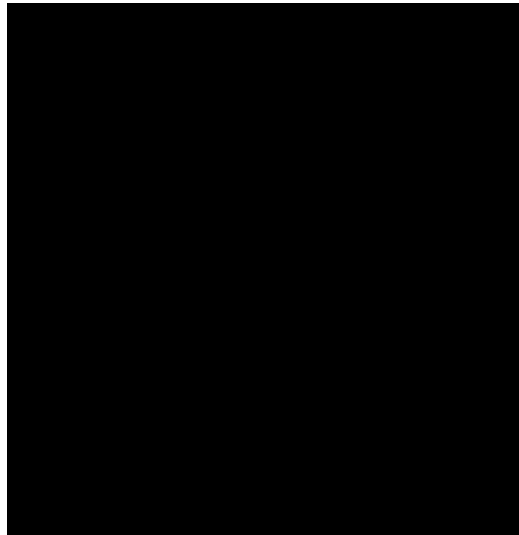


Fig. 57 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 40, approximately 153 bp fragment)  
A1: Ladder Marker, B1: PMO-14 at 600 nmol/L (replicate 3)

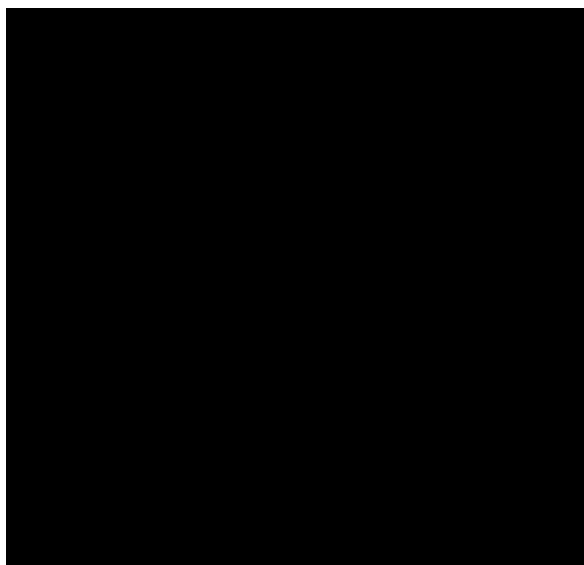


Fig. 58 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 100, approximately 365 bp fragment)  
A1: Ladder Marker, B1: control of PMO-14 (replicate 1), B1: PMO-14 at 300 nmol/L (replicate 3)

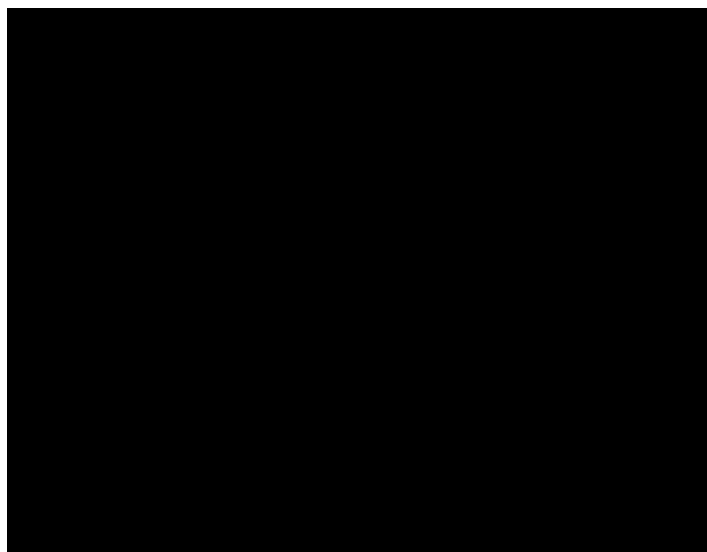


Fig. 59 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 60, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1: control of PMO-14 (replicate 2), C1: PMO-14 at 600 nmol/L (replicate 3), D1 and E1: PMO-14 at 10  $\mu$ mol/L (replicate 1 and 2 respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.



Fig. 60 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-14 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1 and C1: PMO-14 at 600 nmol/L (replicate 1 and 2, respectively), D1: PMO-14 at 10  $\mu$ mol/L (replicate 3)

Exclamation mark indicate that a noise peak was detected outside upper or lower marker.

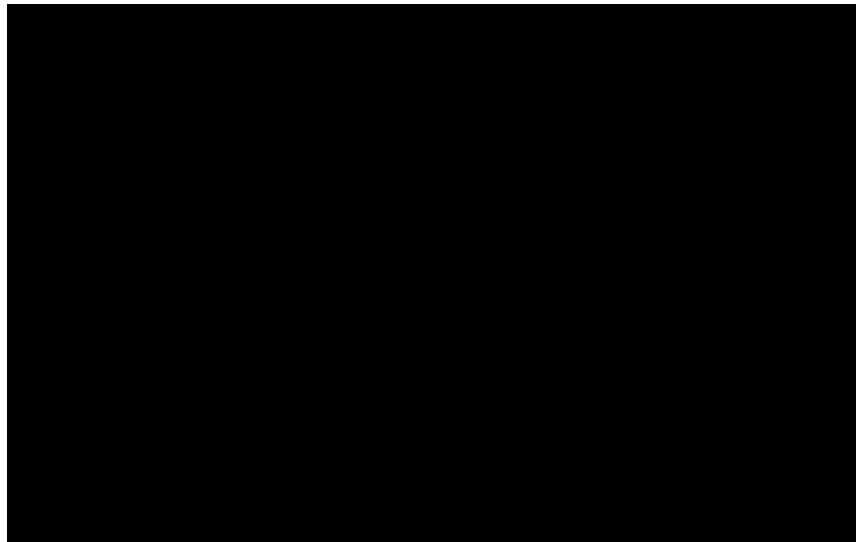


Fig. 61 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-15 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-15 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-15 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-15 at 600 nmol/L (replicate 1 and 2, respectively)

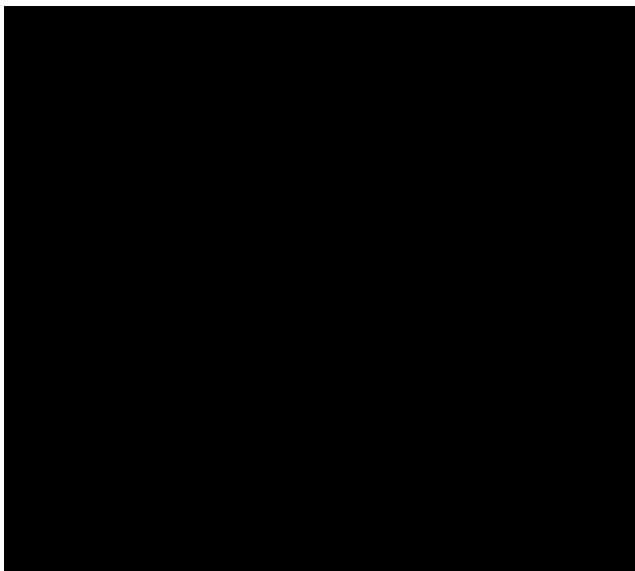


Fig. 62 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-15 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1: PMO-15 at 600 nmol/L (replicate 3)

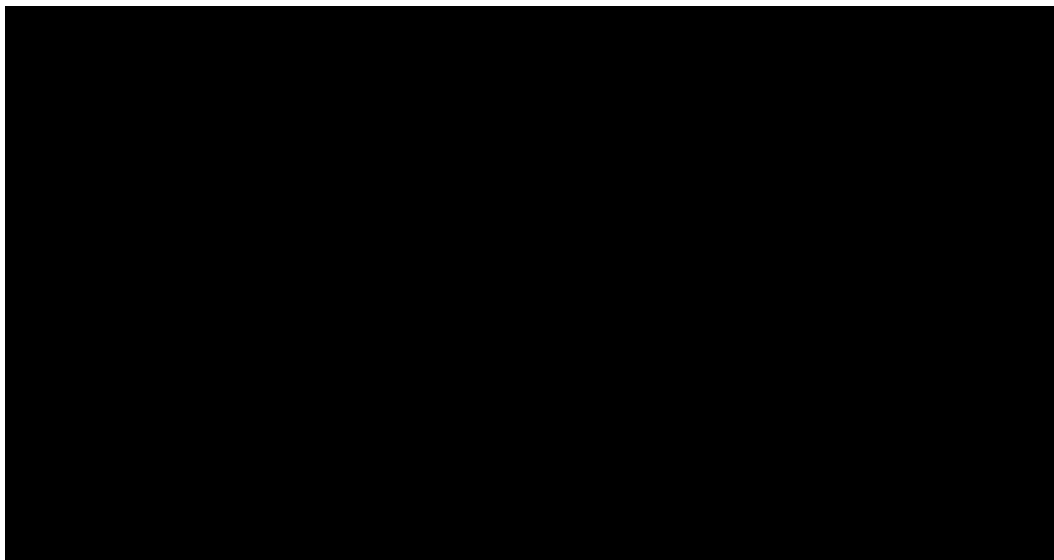


Fig. 63 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-15 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)  
A1: Ladder Marker, B1: control of PMO-15 (replicate 3), C1, D1 and E1: PMO-15 at 300 nmol/L (replicate 1, 2 and 3, respectively), F1 and G1: PMO-15 at 600 nmol/L (replicate 2 and 3, respectively), H1, A2 and B2: PMO-15 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)  
Exclamation marks indicate that a noise peak was detected outside upper or lower marker.





Fig. 64 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-15 exposed cells (Dilution factor 100, approximately 365 bp fragment)

A1: Ladder Marker, B1: control of PMO-15 (replicate 1)

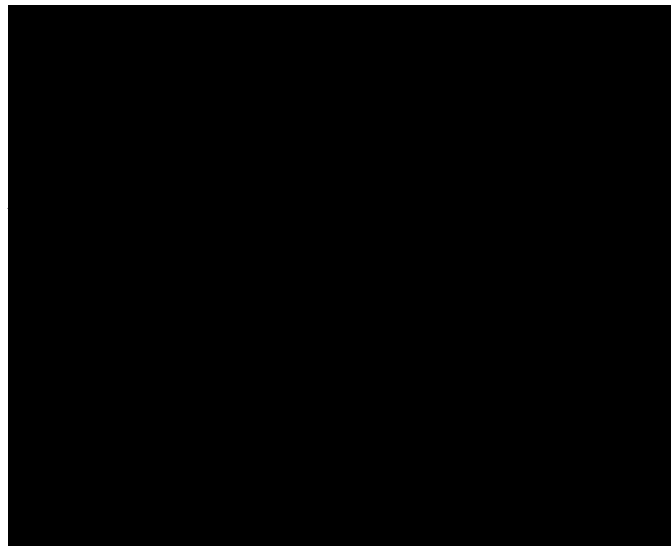


Fig. 65 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-15 exposed cells (Dilution factor 60, approximately 365 bp fragment)

A1: Ladder Marker, B1: control of PMO-15 (replicate 2), C1: PMO-4 at 600 nmol/L (replicate 1)

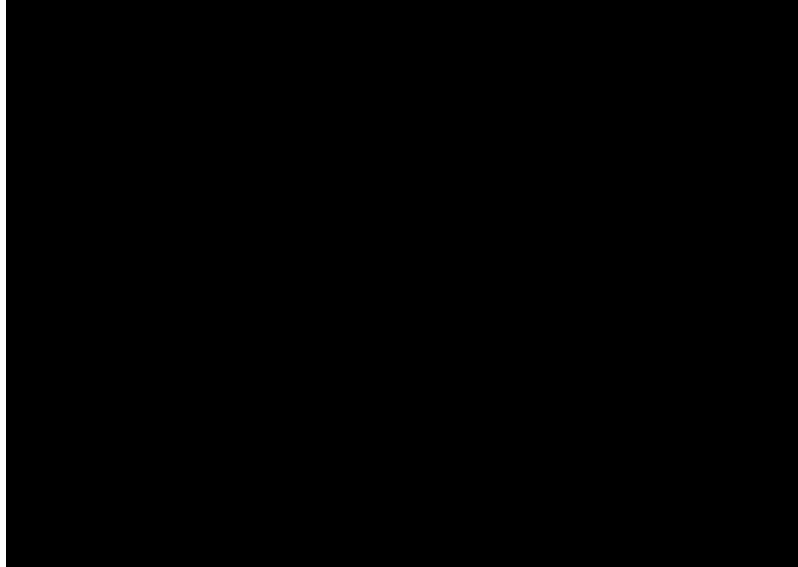


Fig. 66 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-16 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-16 (replicate 1, 2 and 3, respectively), E1: PMO-16 at 300 nmol/L (replicate 3)

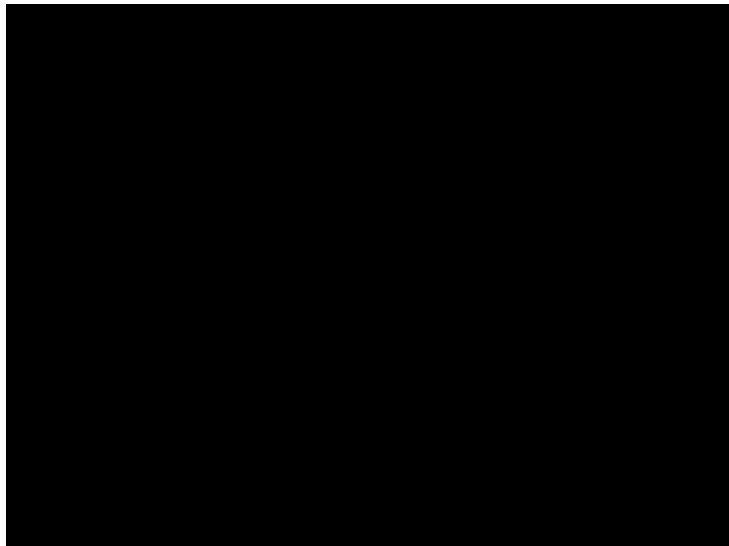


Fig. 67 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-16 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-16 (replicate 1, 2 and 3, respectively)

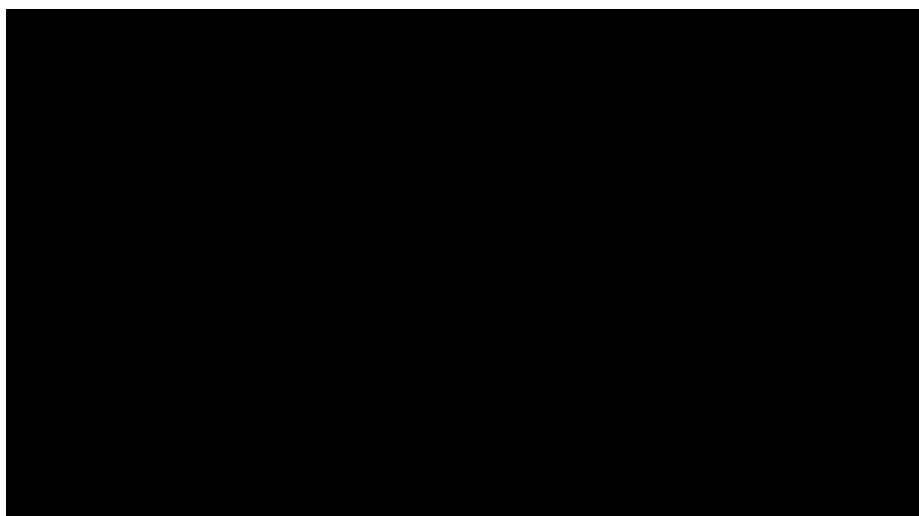


Fig. 68 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-16 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-16 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-16 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-16 at 10  $\mu$ mol/L (replicate 1 and 2, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

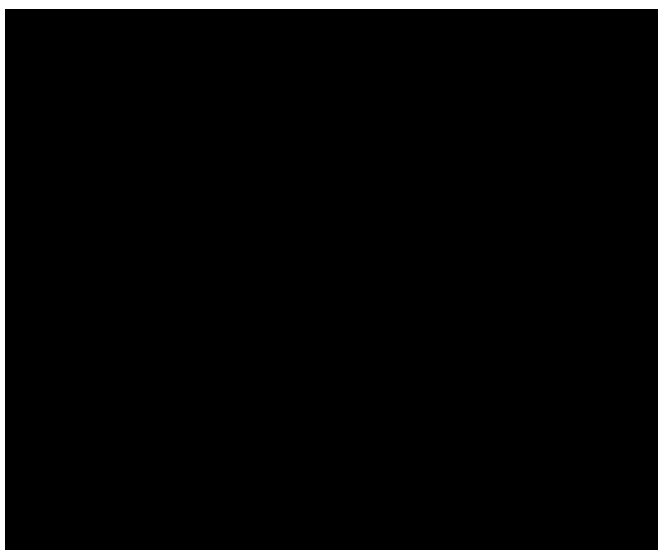


Fig. 69 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-16 exposed cells (Dilution factor 60, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1: PMO-16 at 10  $\mu$ mol/L (replicate 3)

Exclamation mark indicate that a noise peak was detected outside upper or lower marker.

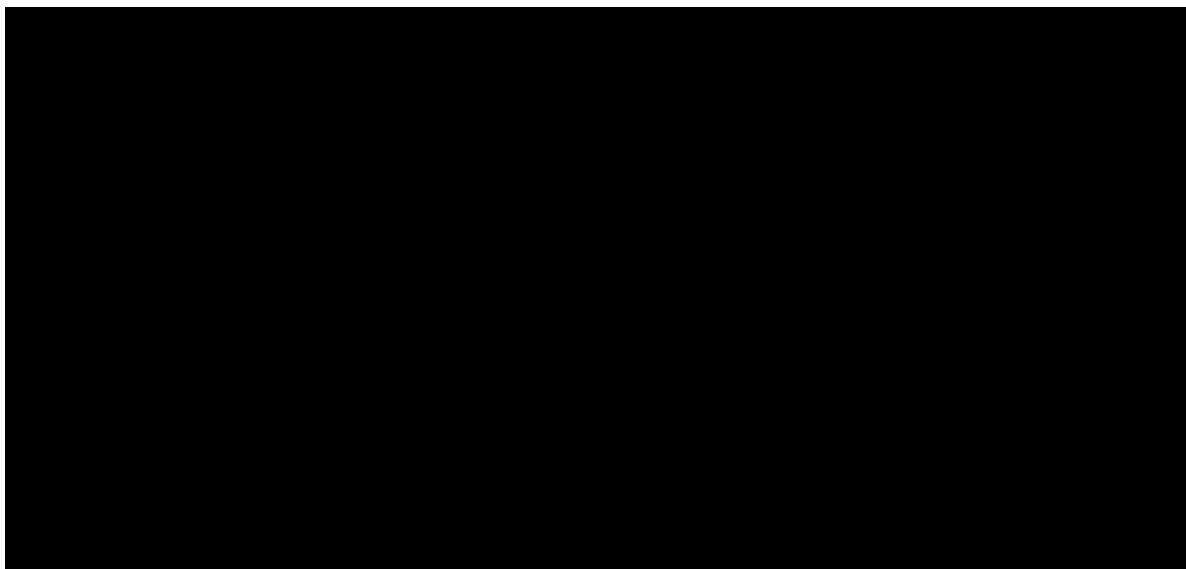


Fig. 70 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-17 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-17 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-17 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-17 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-17 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

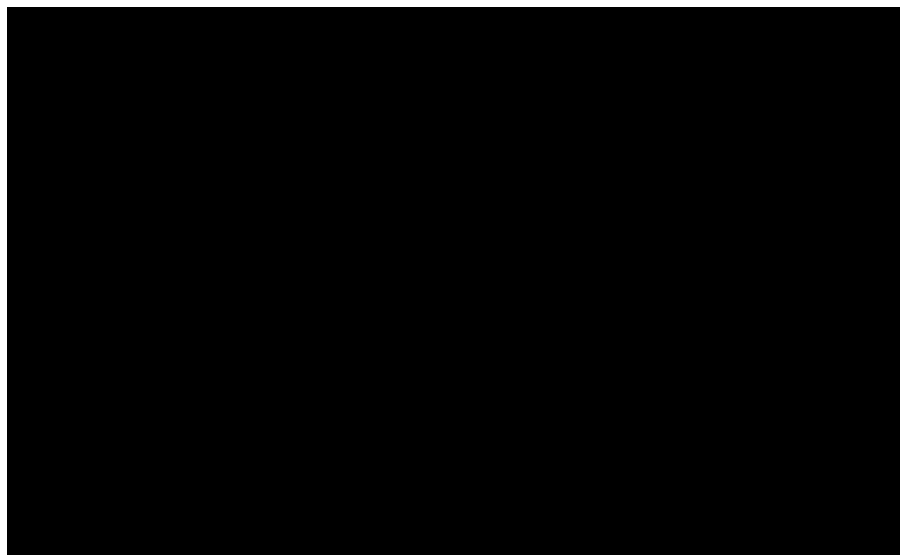


Fig. 71 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-17 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-17 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-17 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-17 at 600 nmol/L (replicate 1 and 2, respectively)

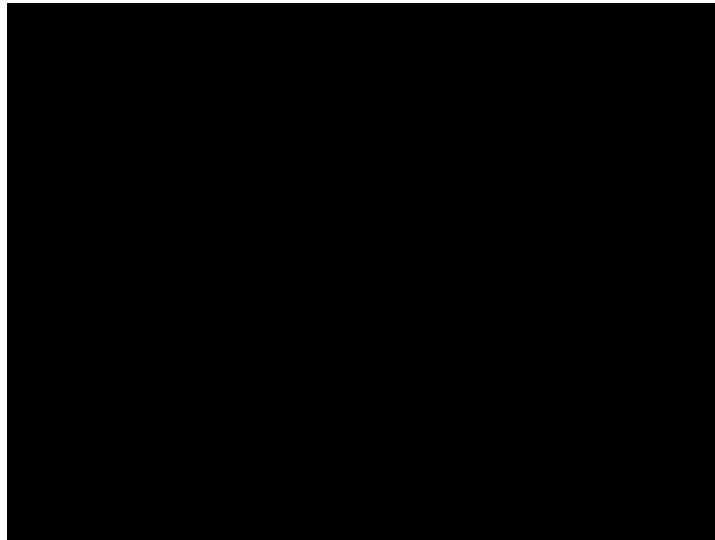
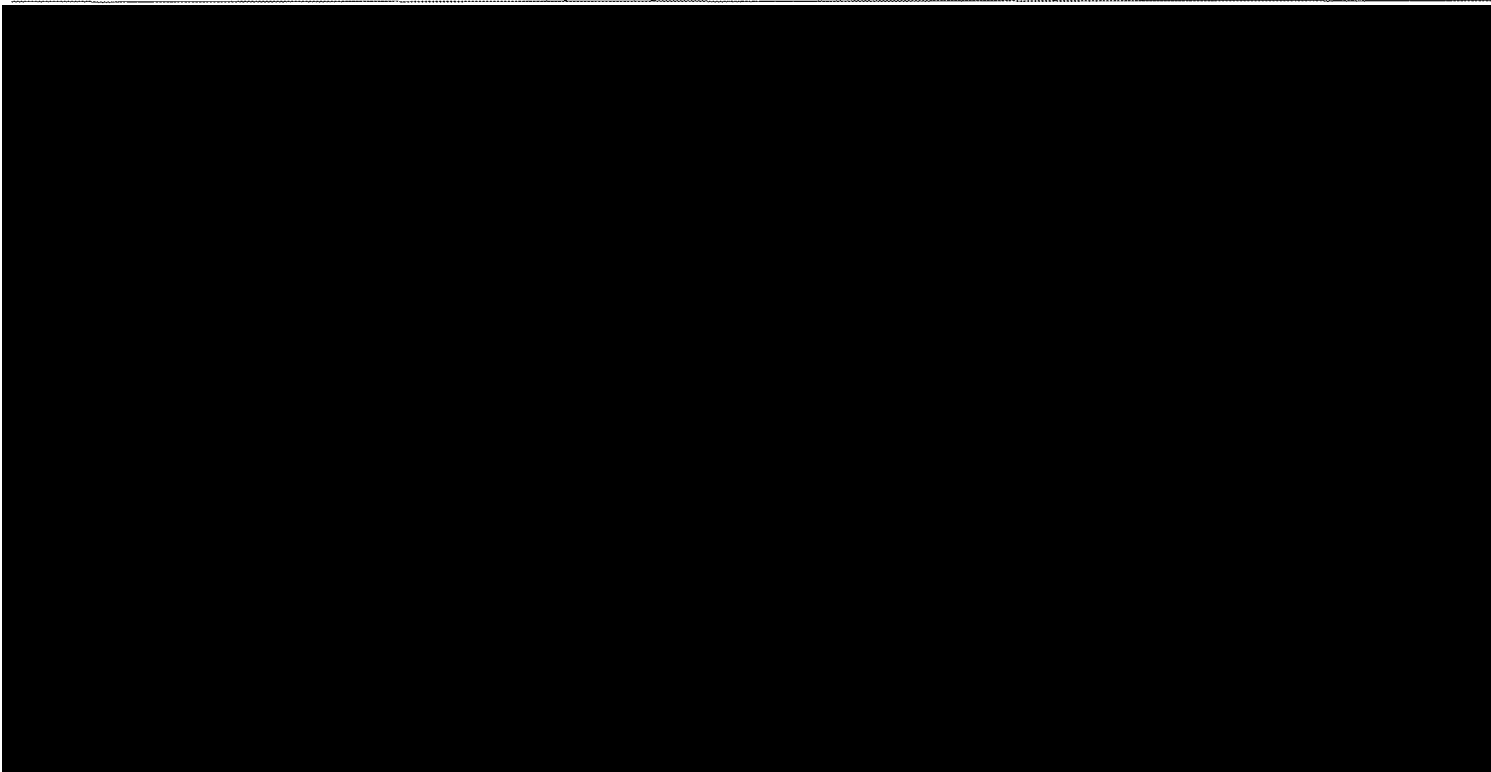
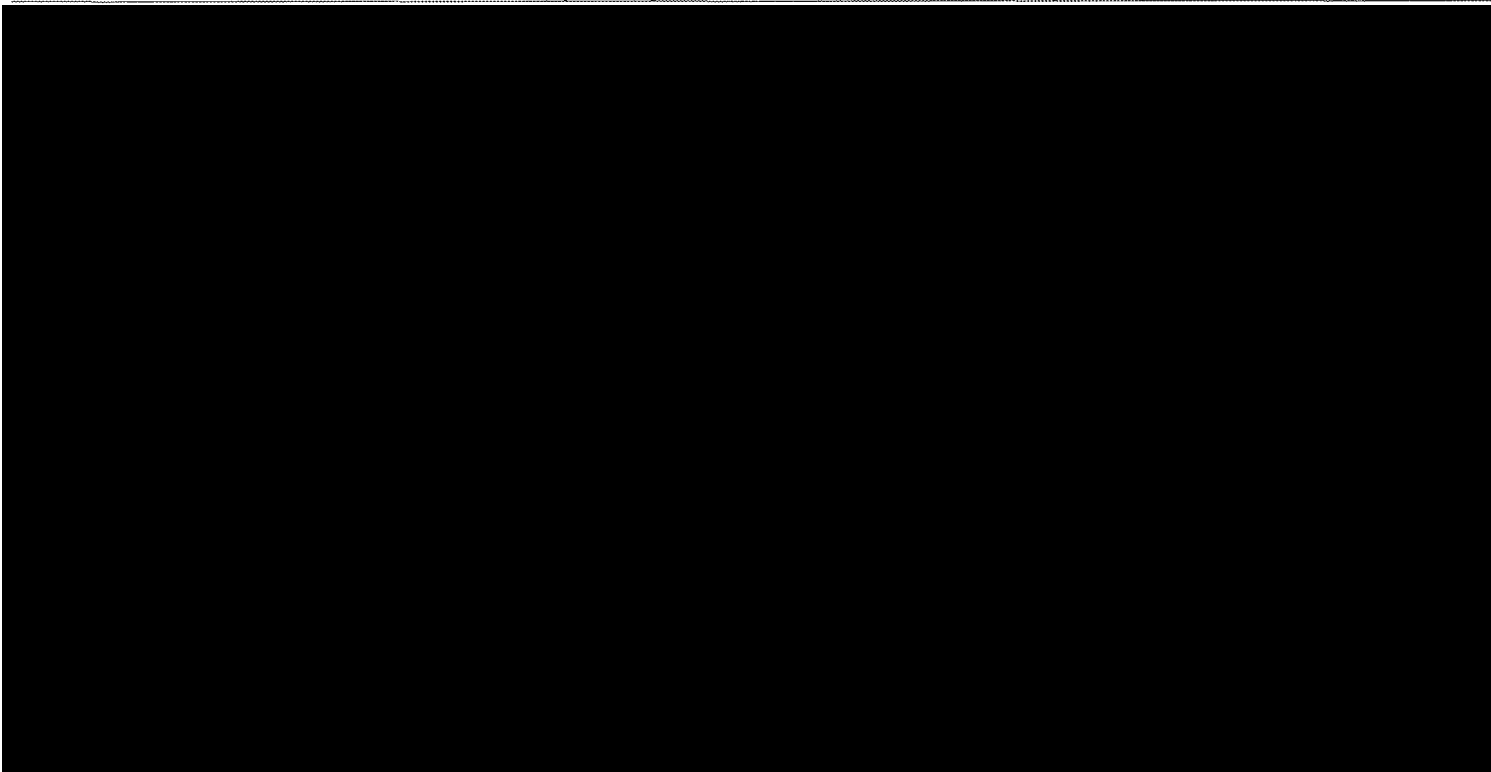
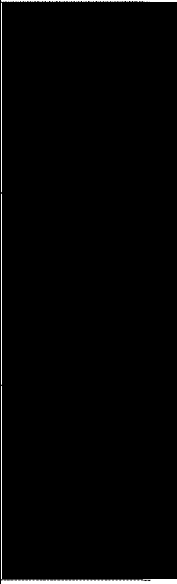


Fig. 72 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-17 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-17 at 600 nmol/L (replicate 3), C1, D1 and E1: PMO-17 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

936-21-M-0644



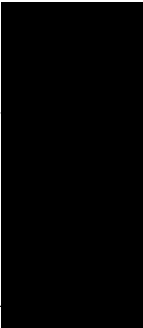
Table 19 Exon skipping efficiency by 4200TapeStation (PMO-2 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

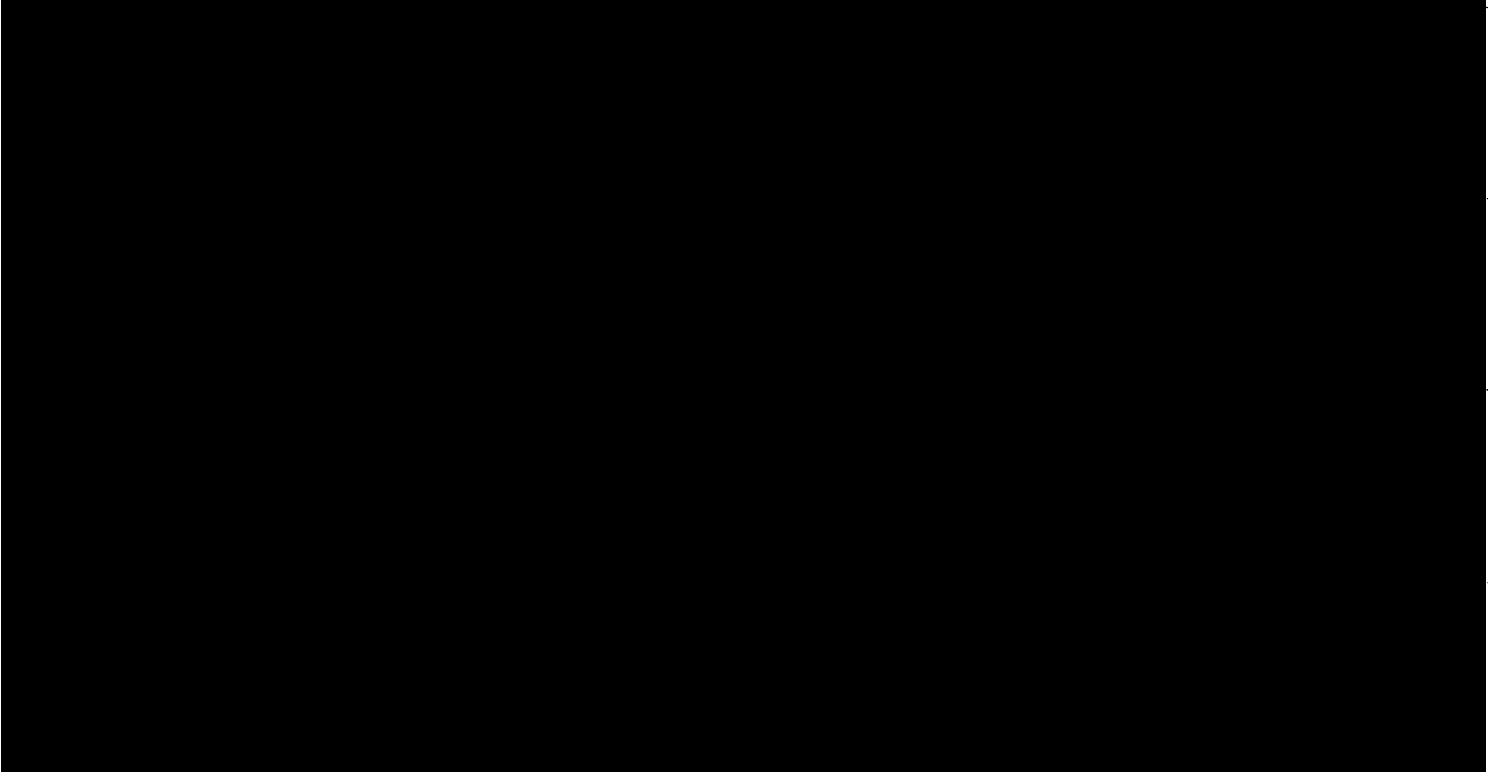

Table 20 Exon skipping efficiency by 4200TapeStation (PMO-3 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

Table 21 Exon skipping efficiency by 4200TapeStation (PMO-4 exposed cells)

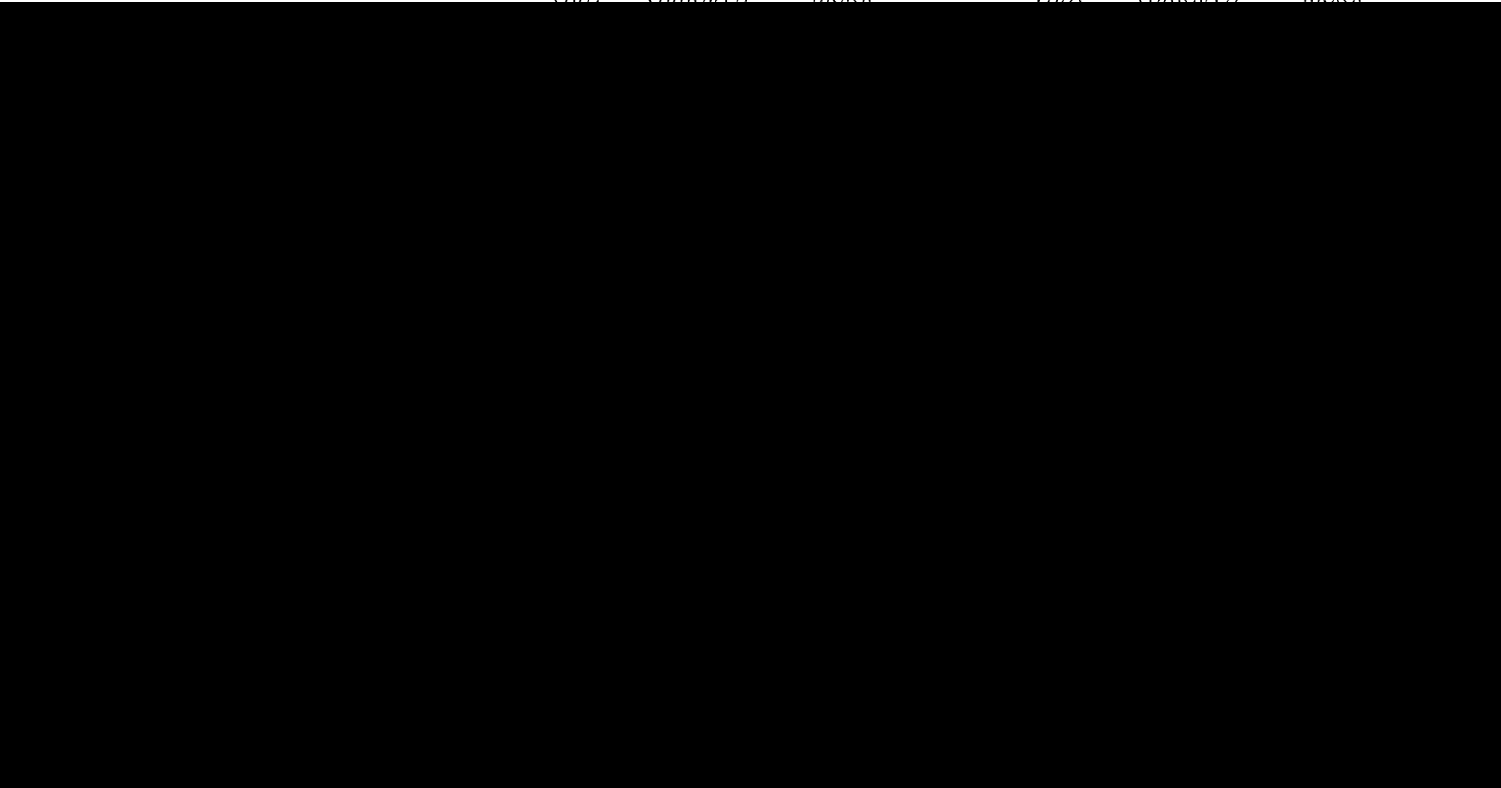
Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable



936-21-M-0644

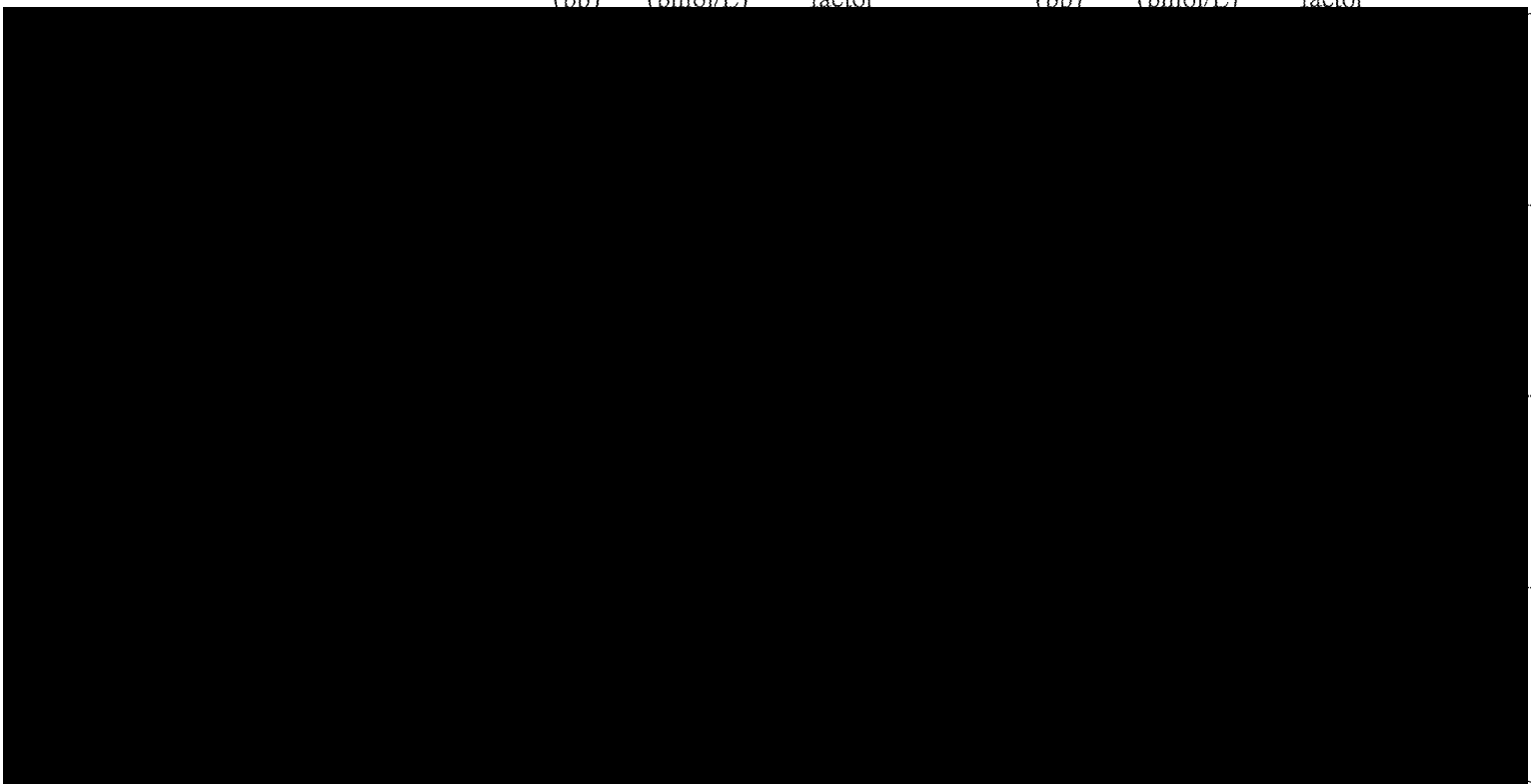
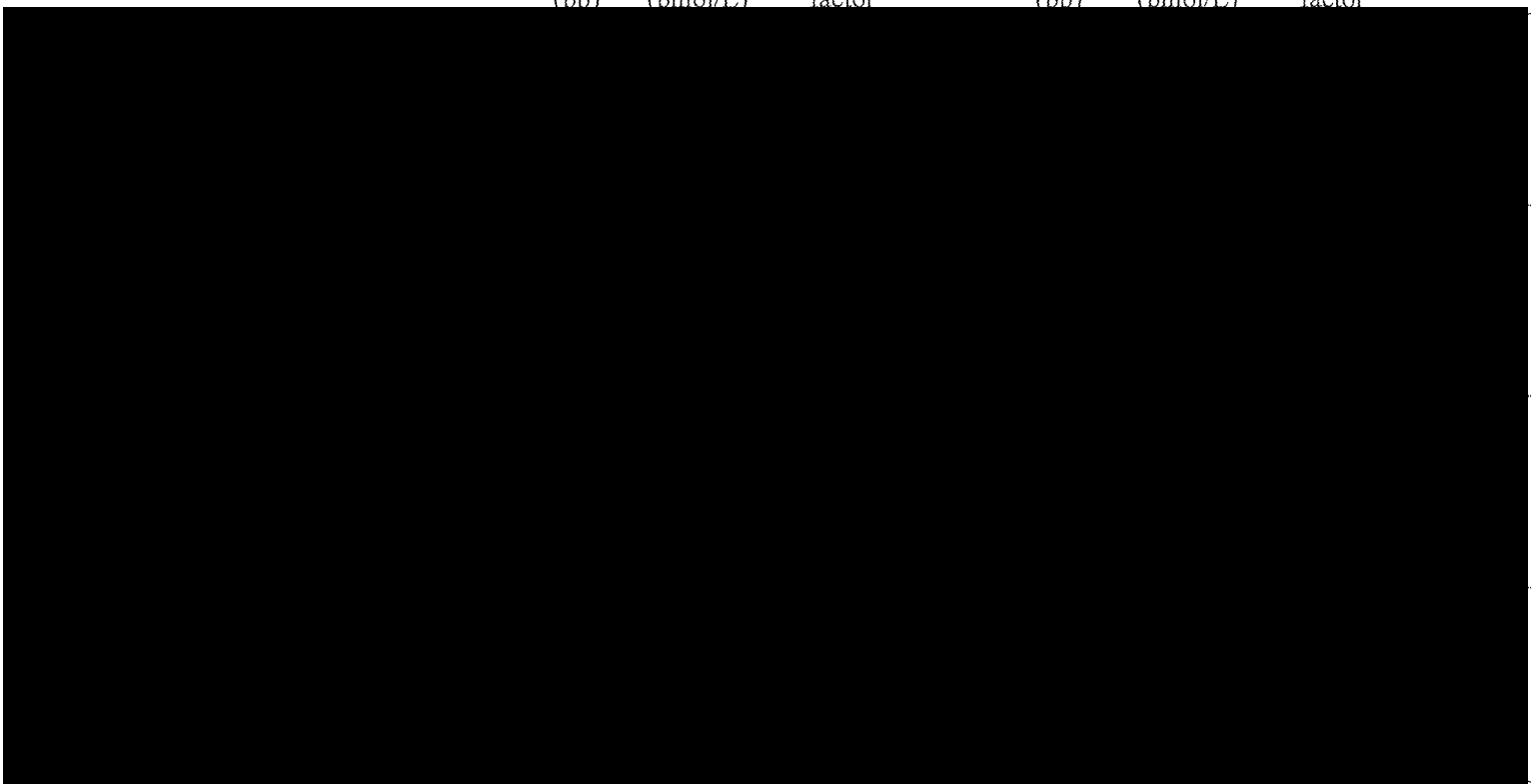


Table 22 Exon skipping efficiency by 4200TapeStation (PMO-5 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

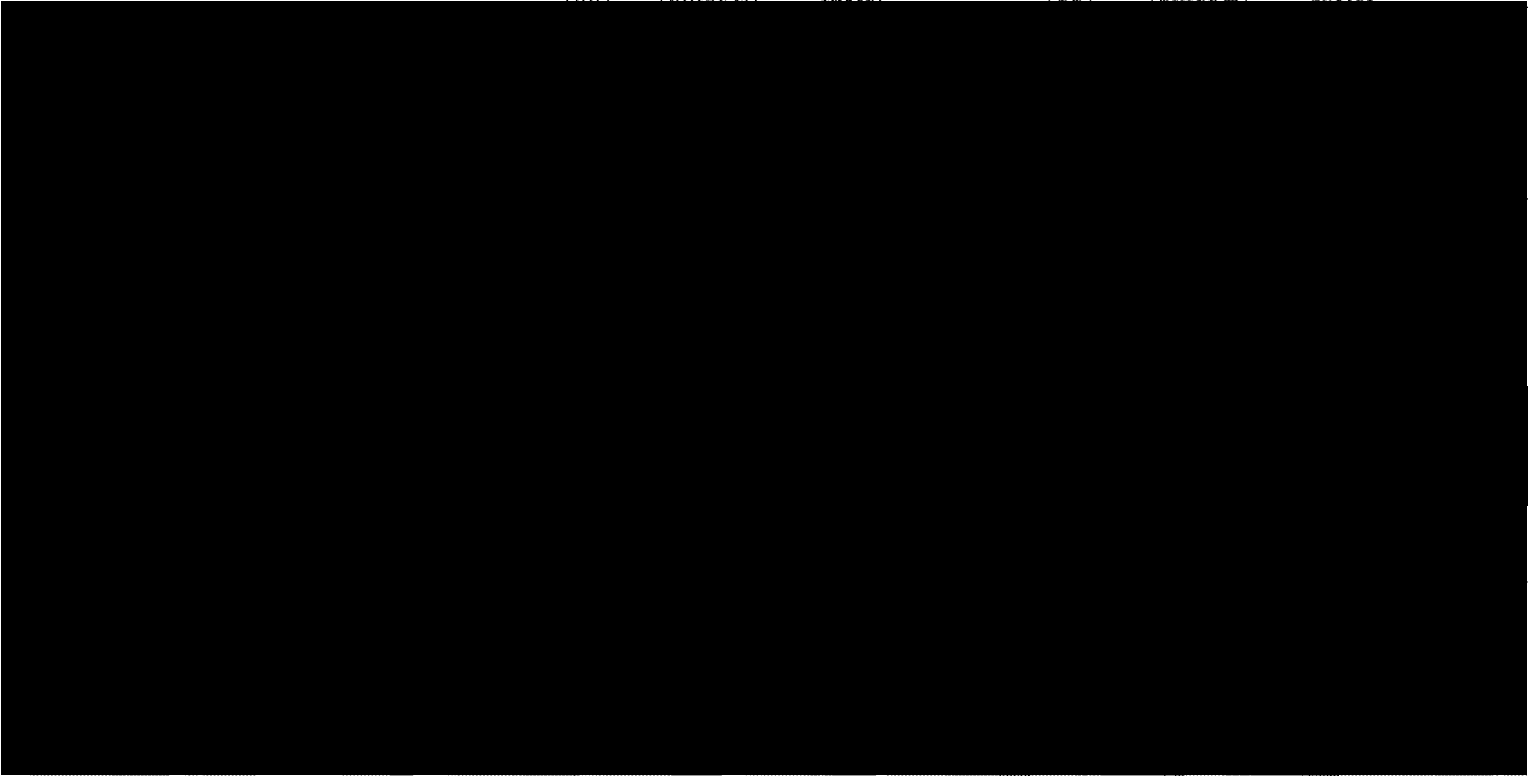
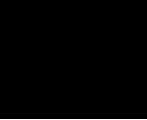

Table 23 Exon skipping efficiency by 4200TapeStation (PMO-6 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											
											
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

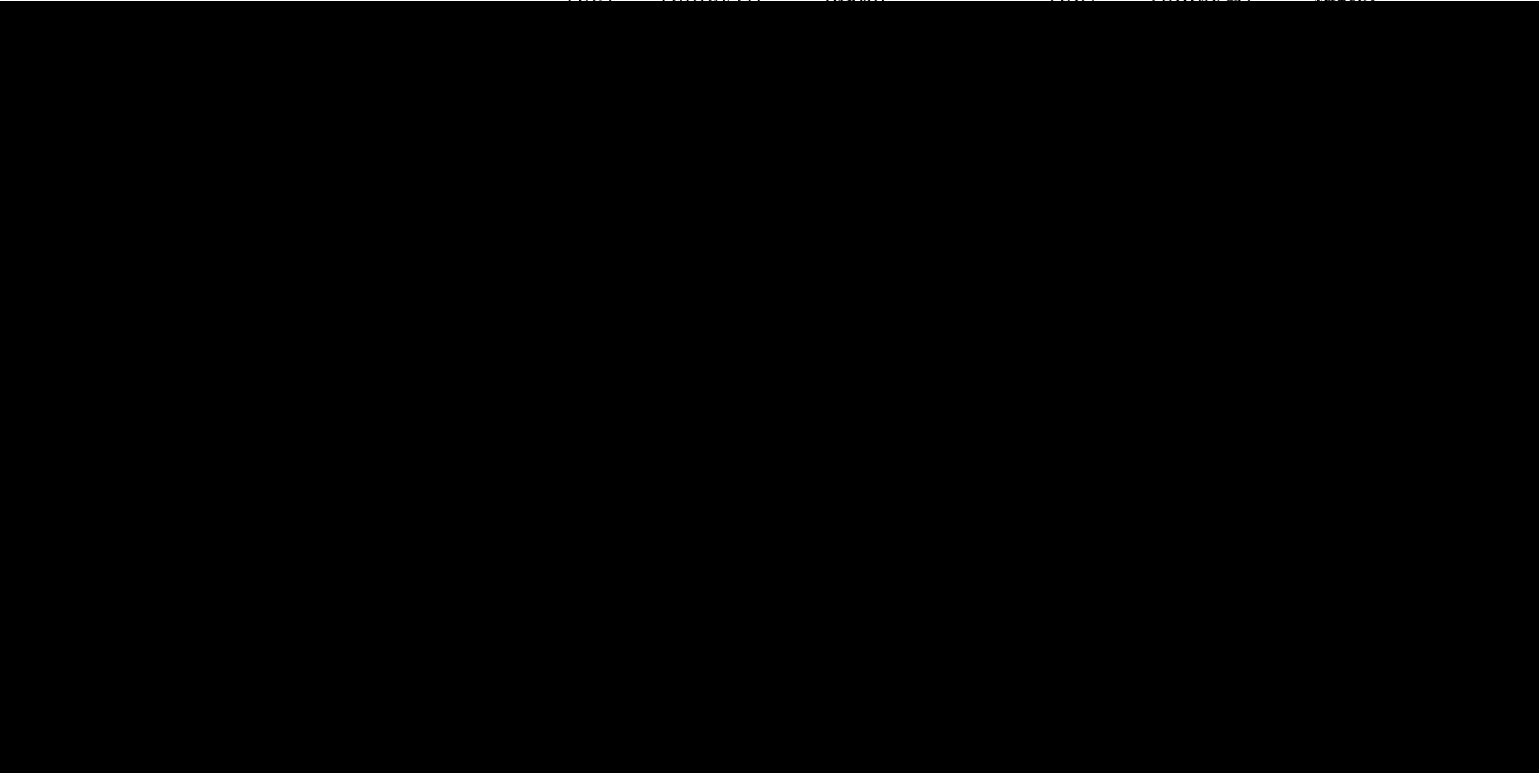
Table 24 Exon skipping efficiency by 4200TapeStation (PMO-7 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											0.1
											N/A
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

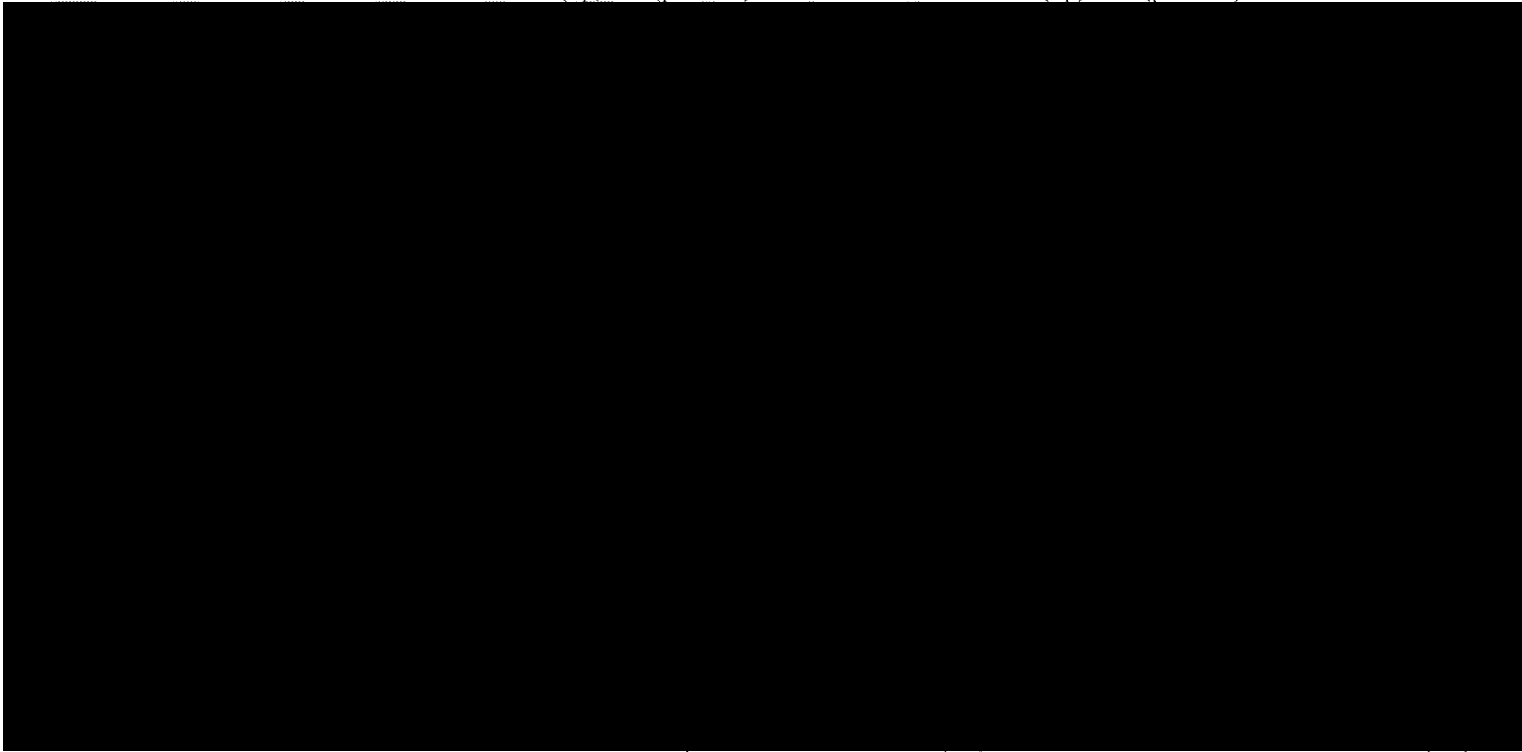
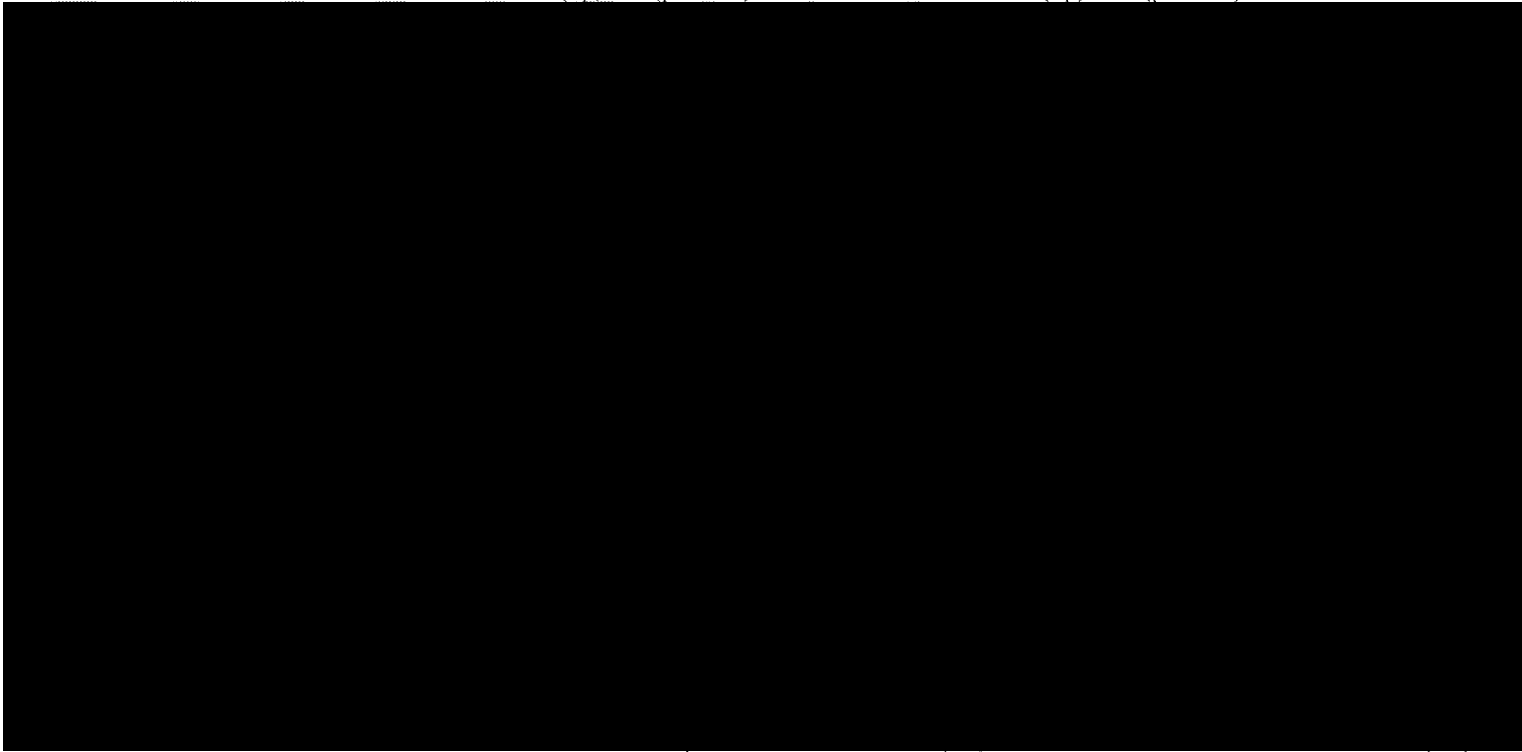

Table 25 Exon skipping efficiency by 4200TapeStation (PMO-8 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

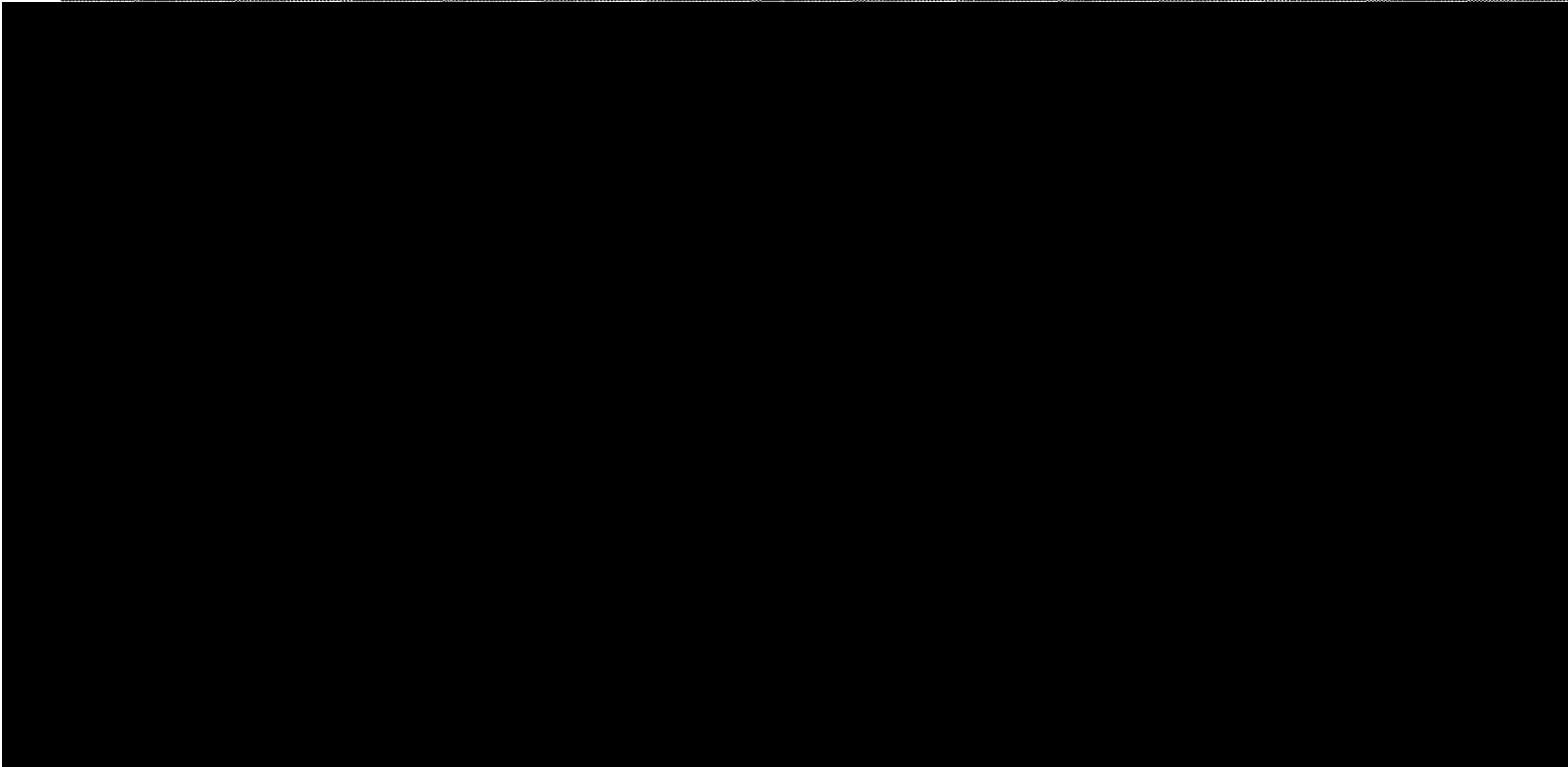
Table 26 Exon skipping efficiency by 4200TapeStation (PMO-9 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

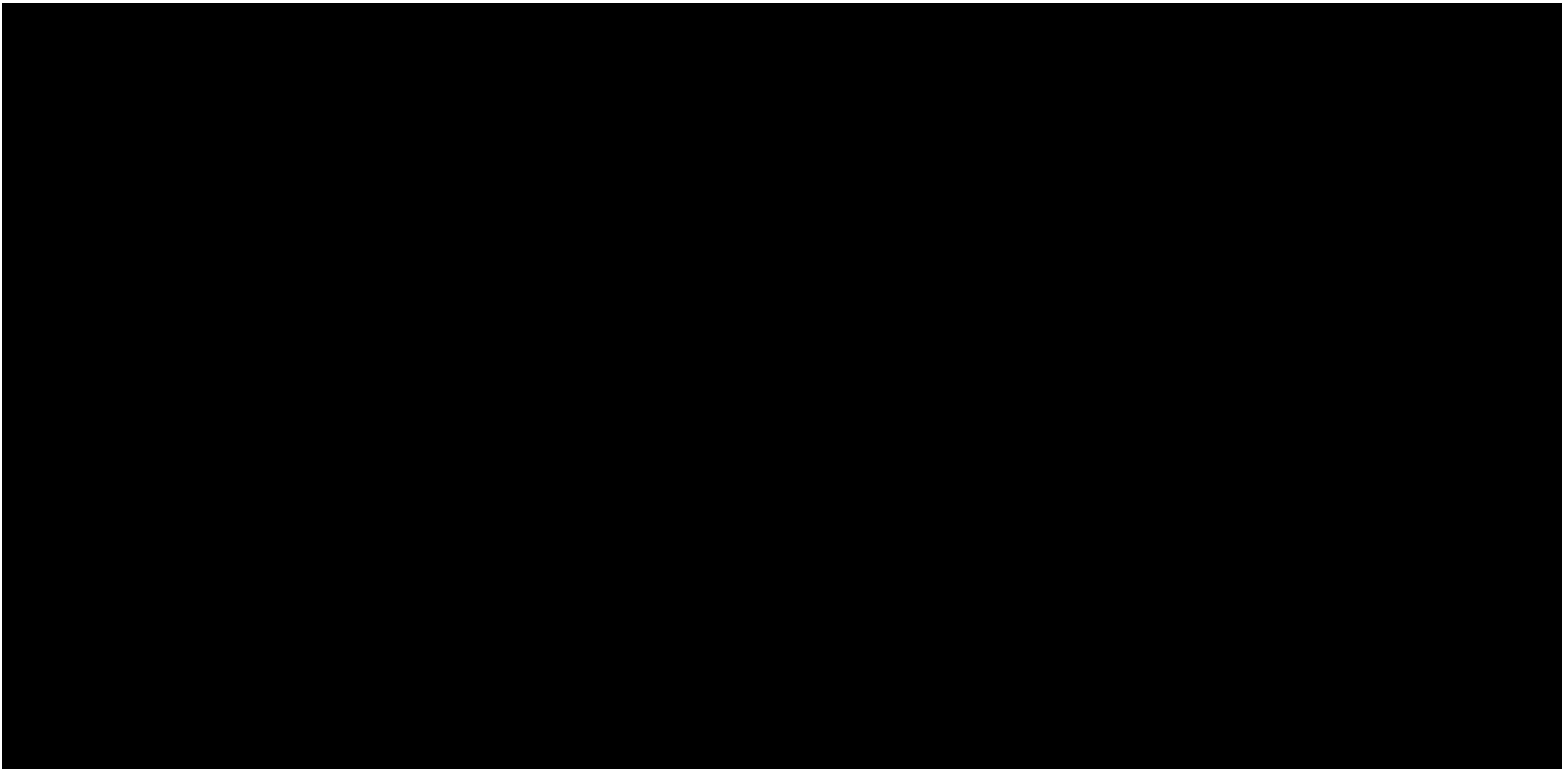

Table 27 Exon skipping efficiency by 4200TapeStation (PMO-10 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

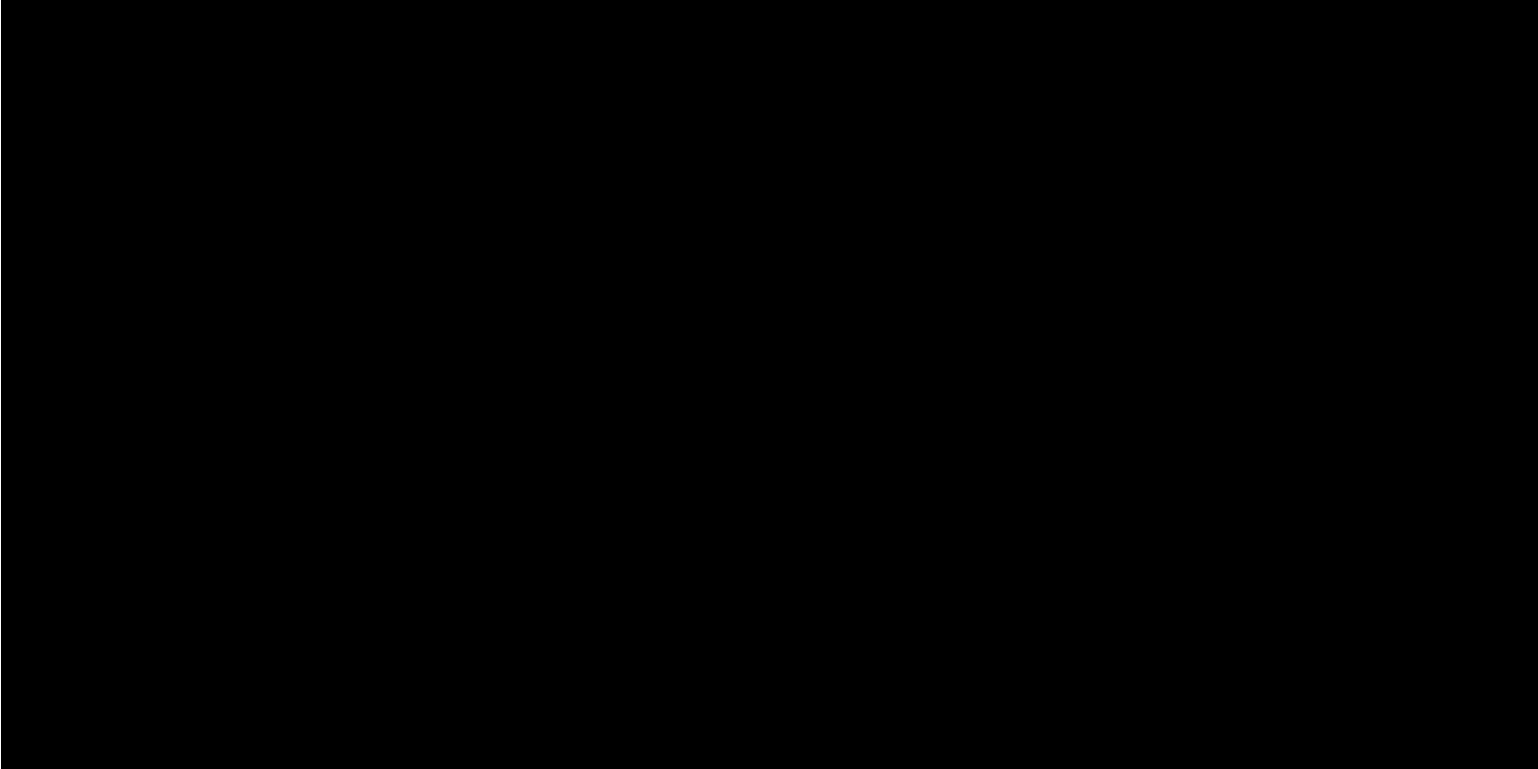




Table 28 Exon skipping efficiency by 4200TapeStation (PMO-11 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											
											N/A

n.d.: not detected, N/A: Not Applicable

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Table 29 Exon skipping efficiency by 4200TapeStation (PMO-12 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											
											
											N/A
											
											

n.d.: not detected, N/A: Not Applicable



936-21-M-0644

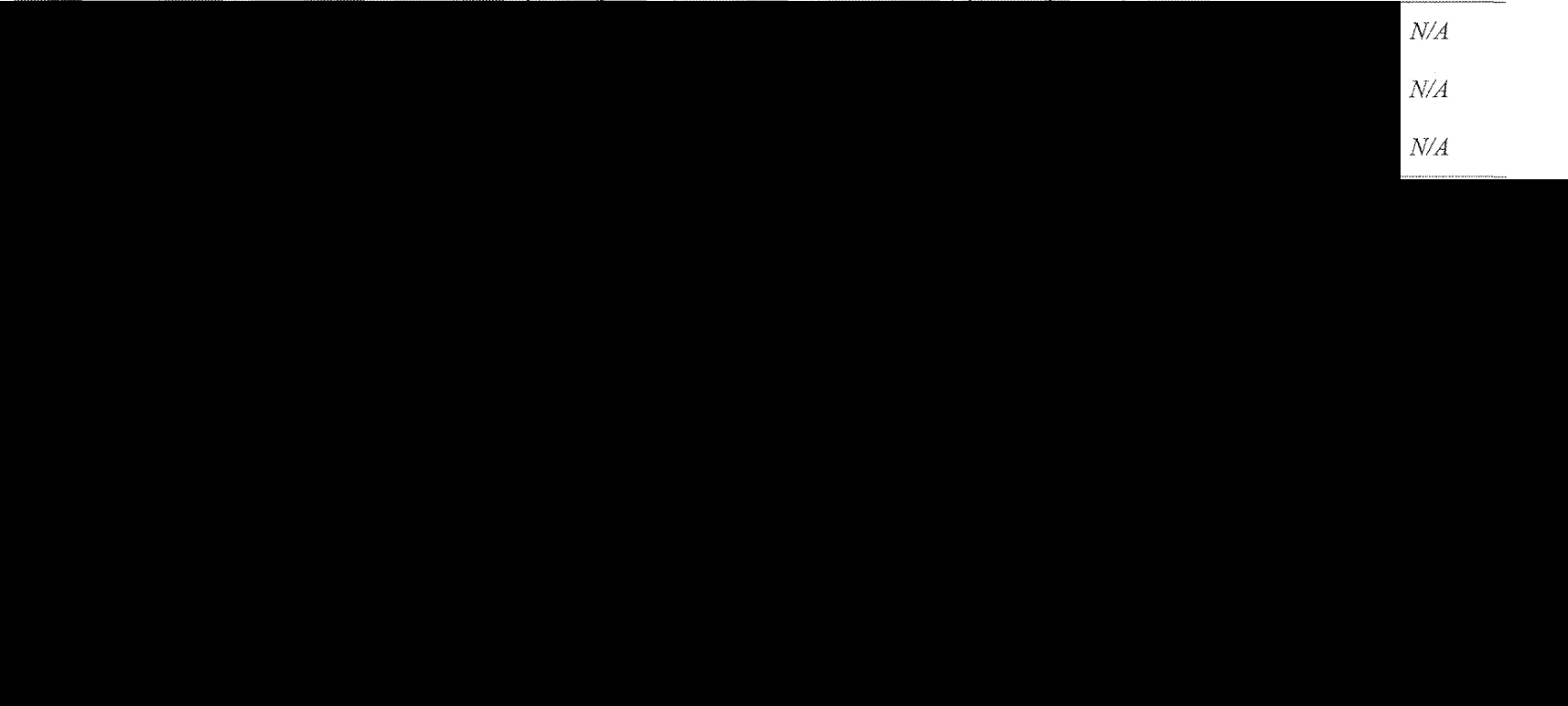
Table 30 Exon skipping efficiency by 4200TapeStation (PMO-13 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency

n.d.: not detected, N/A: Not Applicable

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Table 31 Exon skipping efficiency by 4200TapeStation (PMO-14 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

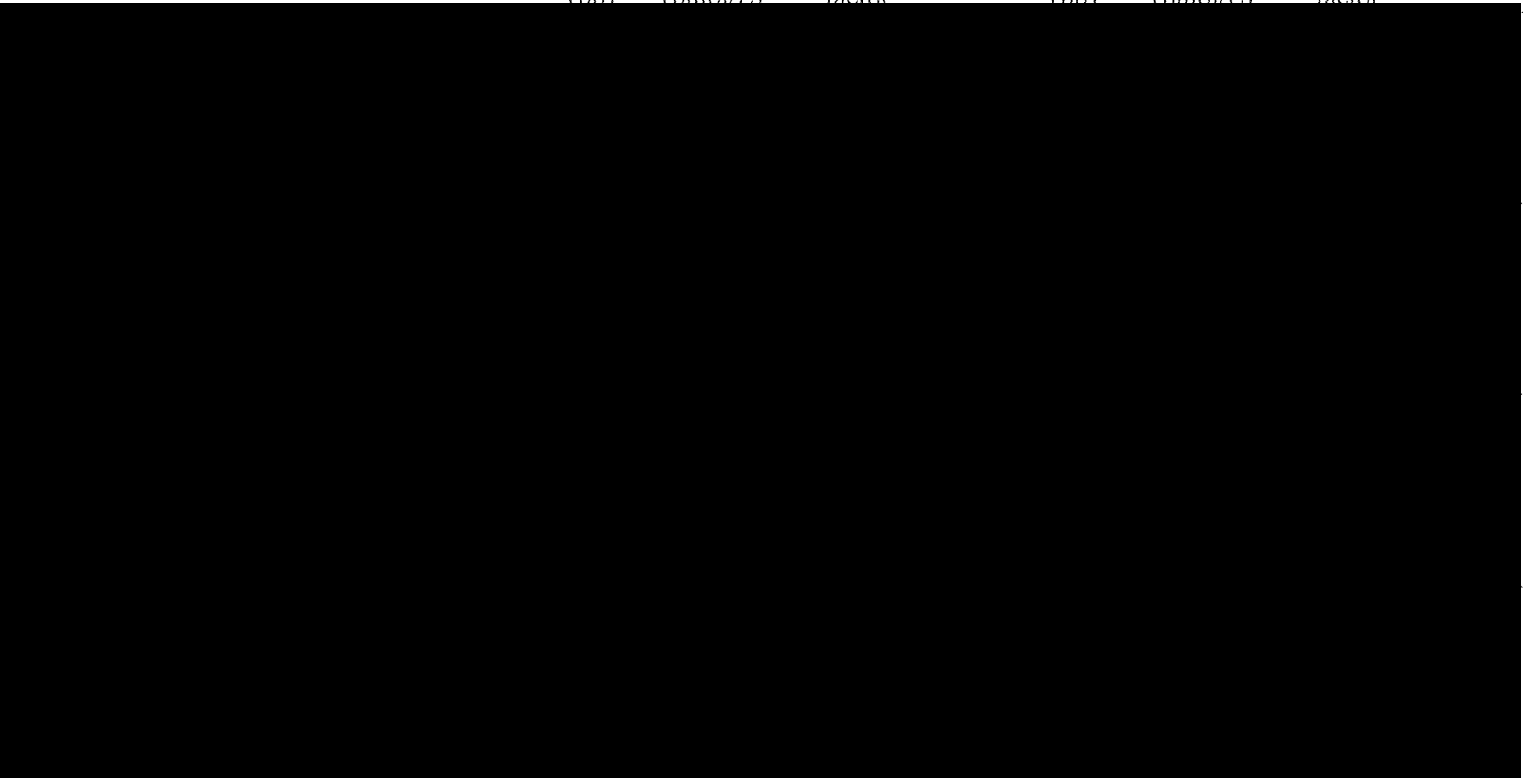
Table 32 Exon skipping efficiency by 4200TapeStation (PMO-15 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

936-21-M-0644

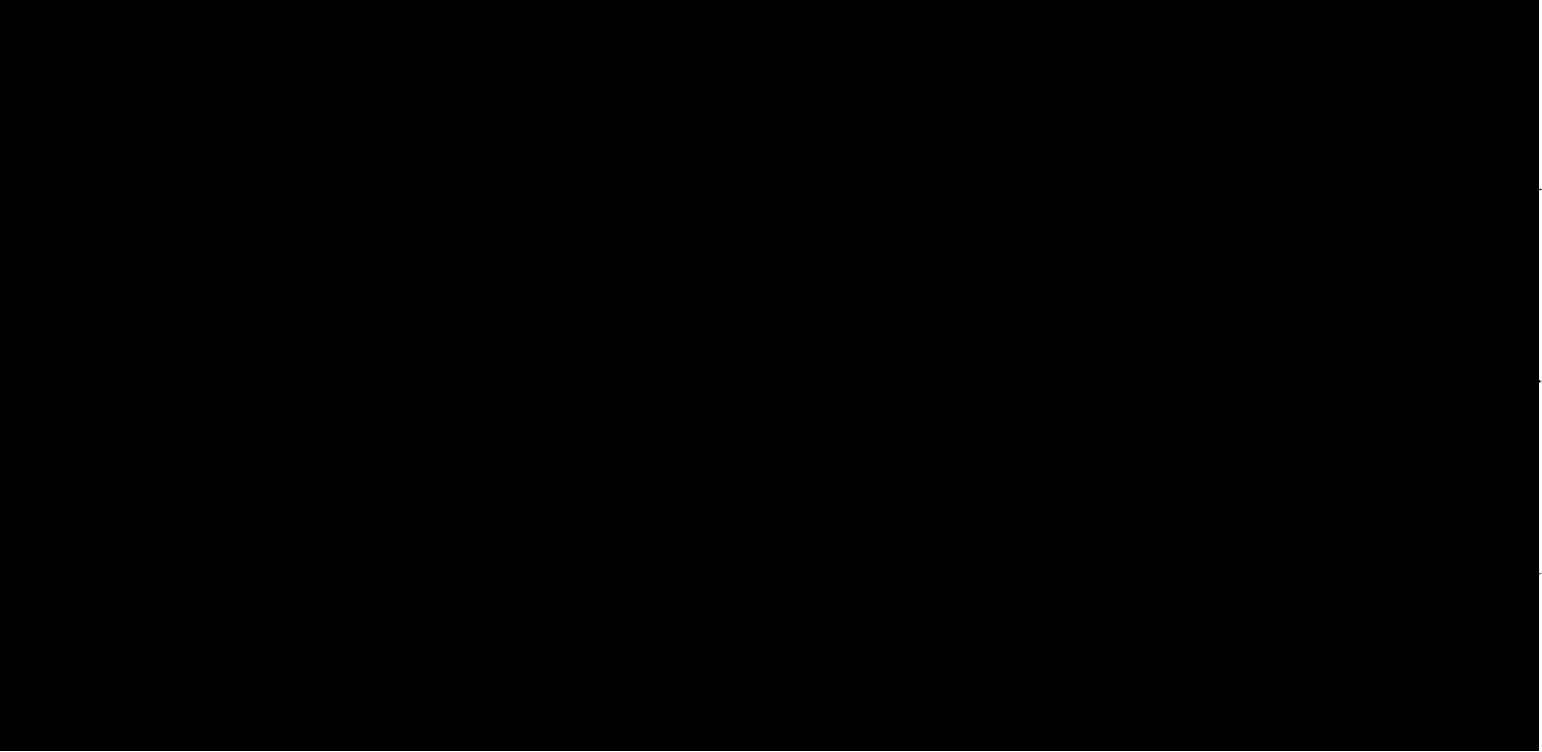
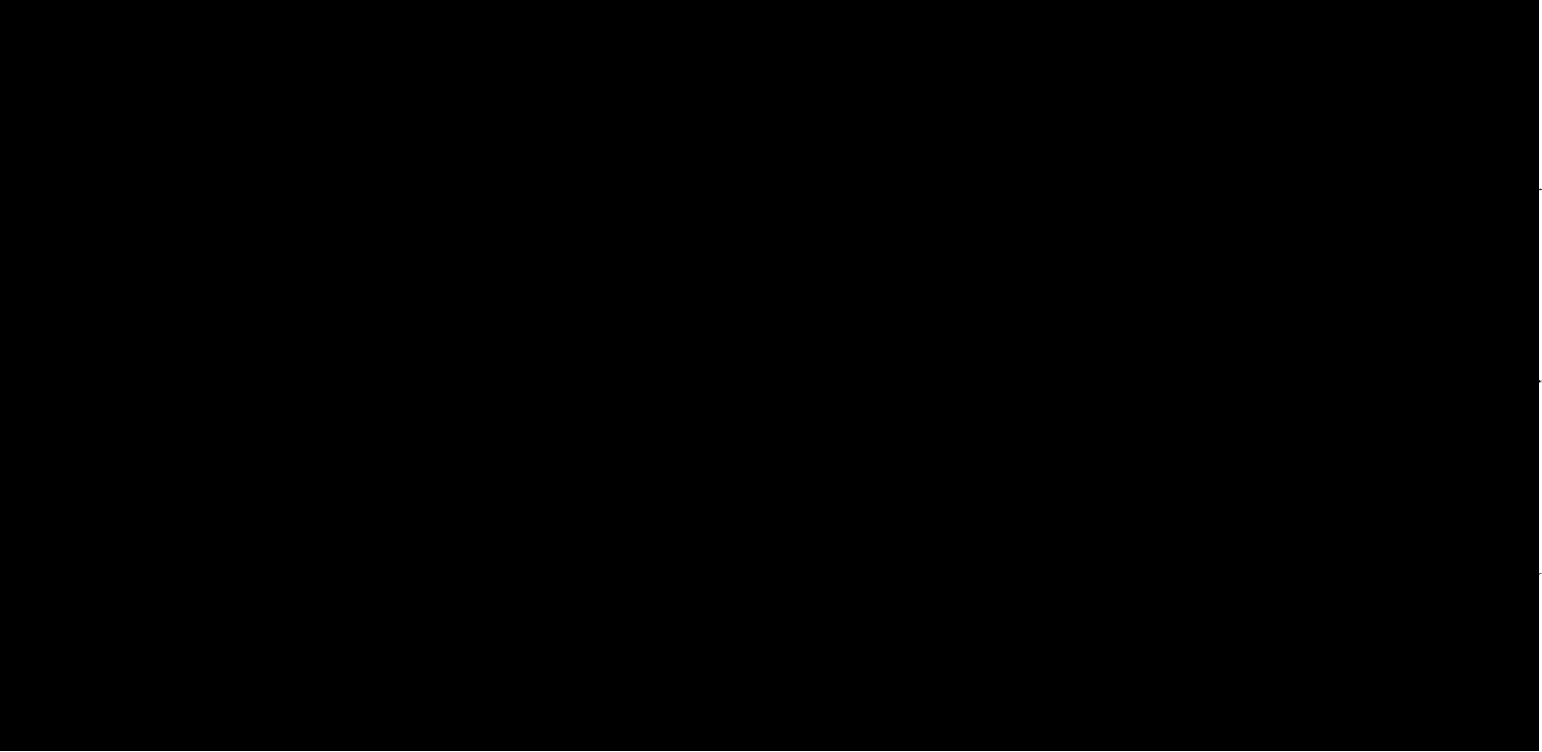
Table 33 Exon skipping efficiency by 4200TapeStation (PMO-16 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

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Table 34 Exon skipping efficiency by 4200TapeStation (PMO-17 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

9. STORAGE OF STUDY PLAN AND FINAL REPORT

The original study plan and final report are one copy and stored in the archives room (4207) of the testing facility. The duplicates of study plan and final report as PDF files are sent to the sponsor.

10. STORAGE AND RETENTION OF DOCUMENT AND RECORD

The original study plan, the original study plan amendment, the original final report, and other records concerning the study are stored in the archives room (4207) of the testing facility for 5 years after the study completion date. The test substances are stored in the archives room (4207) of the testing facility for 1 year after the study completion date. The management (disposal or return) of these items after the storage period will be notified the sponsor in writing before the completion of the storage period. After the storage period, a notice will be given in writing to the sponsor and the stored materials will be returned or disposed.

# Exhibit C



Study Number	936-22-M-0661
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## FINAL REPORT

The assessment of exon skipping activities by PMO in myotube using Endo-Porter DMSO  
as a transfection reagent.

May 2023

Chemicals assessment and research center  
Chemicals Evaluation and Research Institute, Japan (CERI)



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1. TITLE

The assessment of exon skipping activities by PMO in myotube using Endo-Porter DMSO as a transfection reagent.

2. TEST NUMBER

936-22-M-0661

3. OBJECTIVE

To assess exon skipping activities of nine different PMO in myotube.

4. TESTING FACILITY

Name: Chemicals Assessment and Research Center, Chemicals Evaluation and Research Institute, Japan (CERI)

Address: 1600, Shimotakano, Sugito-machi, Kitakatsushika-gun, Saitama 345-0043, Japan

5. PERIOD OF STUDY

Study initiation date.: October 17, 2022

Experimental starting date.: October 19, 2022

Experimental completion date.: December 15, 2022

Study completion date.: May 17, 2023

## 6. MATERIALS AND METHODS

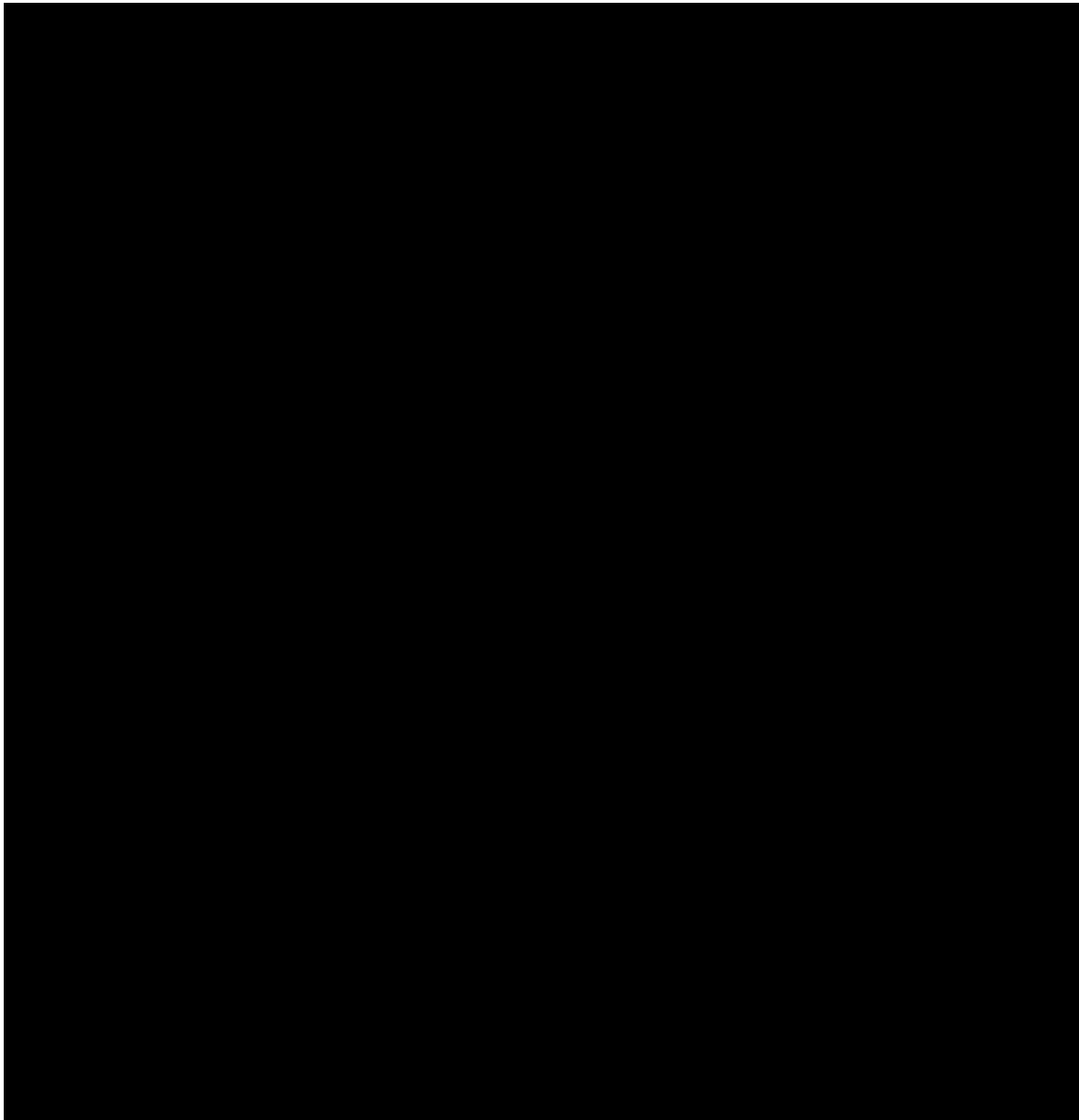
### 6.1 Test substance and vehicle

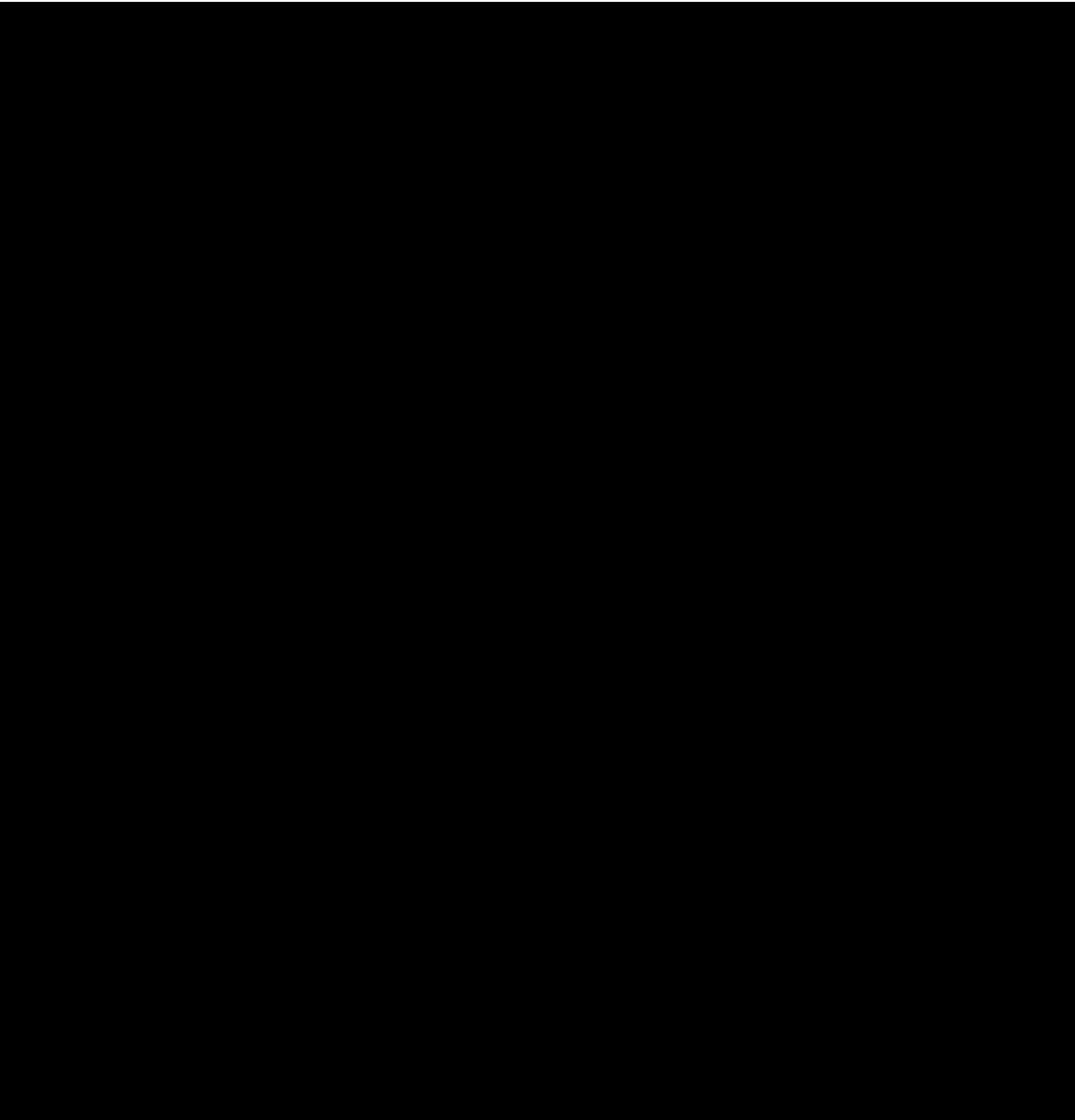
### 6.1.1 Test substances

The test substances to be used in this study were listed in Table 1.

[illegible]

Test substance ID	PMO-R2	PMO-R3	PMO-R4
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Test substance ID	PMO-R5	PMO-R6	PMO-R7
			

Gloves, a mask and a lab coat were worn when handling these substances.

6.1.2 Vehicle control substance

(1) Name

Water for injection

(2) Grade

Japanese Pharmacopoeia

(3) Lot number

K2D76

(4) Manufacturer

Otsuka Pharmaceutical Factory

(5) Storage condition

Vehicle was stored at room temperature.

(6) Handling precautions

Gloves, a mask, and a lab coat were worn when handling.

6.2 Cells

Name:

Supplier:

Lot number:

6.3 Reagents, equipments and softwares

(1) Reagents

- PBS (-) powder (Thermo Fisher Scientific)
- SkGM™-2 BulletKit™ (Lonza)
- Trypsin (Thermo Fisher Scientific)
- Dulbecco's Modified Eagle Medium: F12 (DMEM: F12) (Lonza)
- Horse serum (Thermo Fisher Scientific)
- Trypan Blue Stain 0.4% (Thermo Fisher Scientific)
- Endo-Porter, DMSO (GENE TOOLS)
- Trizol (Thermo Fisher Scientific)
- 2-Propanol (Molecular Biology, FUJIFILM Wako Pure Chemical)
- Ethanol (Guaranteed Reagent, FUJIFILM Wako Pure Chemical)
- Chloroform (Guaranteed Reagent, FUJIFILM Wako Pure Chemical)
- Water for injection (Grade: Japanese Pharmacopoeia, Otsuka Pharmaceutical Factory)
- QIAshredder (QIAGEN)
- RNeasy PLUS Micro Kit (QIAGEN)

- High Sensitivity RNA ScreenTape (Agilent)
- High Sensitivity RNA Sample Buffer (Agilent)
- High Sensitivity RNA Ladder (Agilent)
- Nuclease Free Water (Thermo Fisher Scientific)
- Titan One-tube RT-PCR system (Sigma-Aldrich)
- Outer Forward Primer (PS192: 5'-CTT GGA CAG AAC TTA CCG ACT GG-3')
- Outer Reverse Primer (PS197: 5'-GTT TCT TCC AAA GCA GCC TCT CG-3')
- Inner Forward Primer (PS193: 5'-GCA GGA TTT GGA ACA GAG GCG-3')
- Inner Reverse Primer (PS195: 5'-CAT CTA CAT TTG TCT GCC ACT GG-3')
- Tth DNA Polymerase (Sigma-Aldrich)
- PCR Nucleotide Mix (Sigma-Aldrich)
- High SensitivityD1000 ScreenTape (Agilent)
- High Sensitivity D1000 Reagents (Agilent)
- High Sensitivity D1000 Ladder (Agilent)
- TE Buffer, 1X (Molecular Biology Grade, Promega)

(2) Equipments

- CO<sub>2</sub> incubator (SANYO, MCO-175M)
- Biosafety cabinet (DALTON, NSC-II-A-1200 and AIR TECH, BHC-1303 IIA/B3)
- Water bath (Yamato, Thermo-Mate BF400)
- Refrigerated centrifuge (Kubota, 3740)
- Ultrapure water system (Komatsu Electronics, KE0147UC)
- Magnetic stirrer (ADVANTEC, SR500)
- Refrigerator (SANYO, MPR-514 and Panasonic, MPR-162DCN-PJ)
- Freezer (SANYO, MDF-U537D and SANYO, MDF-U332 and SANYO, MDF-U333)
- Deep freezer (Panasonic, MDF-U500VXS5-PJ and SANYO, MDF-U52VS5)
- Automated RNA purification system (QIAGEN, QIAcube)
- Liquid nitrogen tank (CRYOGENIC EQUIPMENT, SR-31)
- NanoDrop One (Thermo Fisher Scientific, ND-ONE-W)
- Micro refrigerated centrifuge (TOMY, MDX-310)
- TapeStation (Agilent, 4200 TapeStation)
- Thermal cycler (Thermo Fisher Scientific, ProFlex)
- Dry bath incubator (BSR-MK10, Bio medical science)

(3) Software

- Excel 2019 (Microsoft)

#### 6.4 Culture medium

- [REDACTED]

##### (1) Composition

[REDACTED]

##### (2) Preparation

[REDACTED]

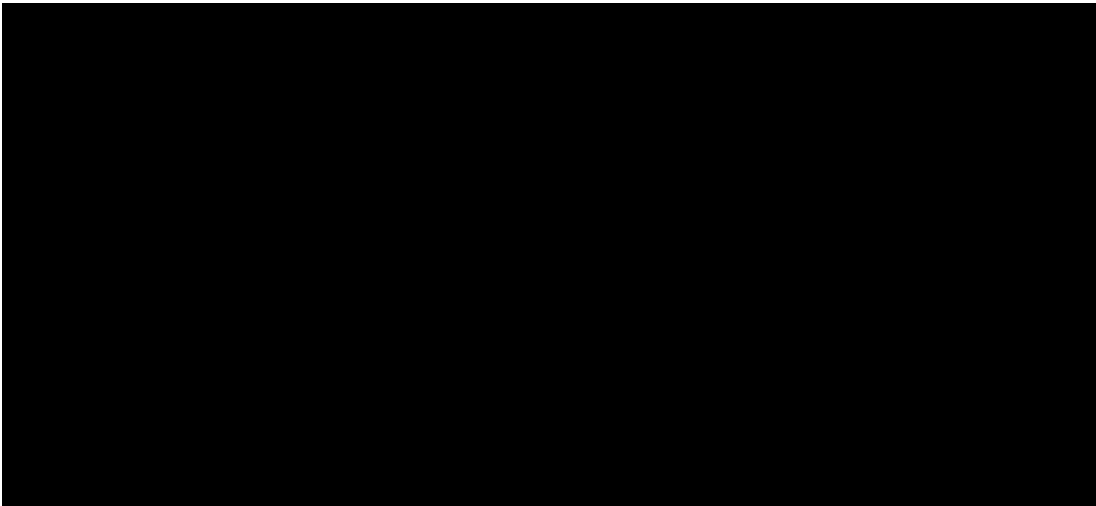
- Differentiation medium (HS-DMEM)

[REDACTED]

#### 6.5 Cell culture and differentiation

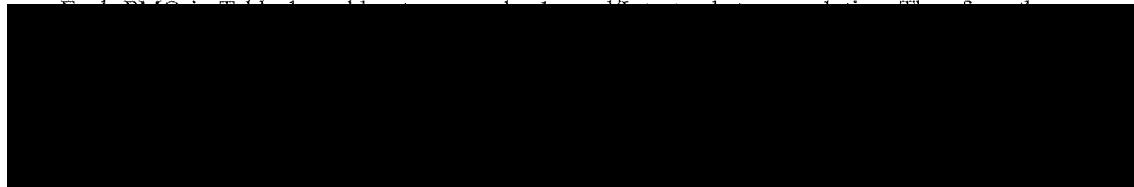
[REDACTED]





6.6 Transfection

6.6.1 Preparation of test substance solution.



6.6.2 Preparation of medium containing test substance.

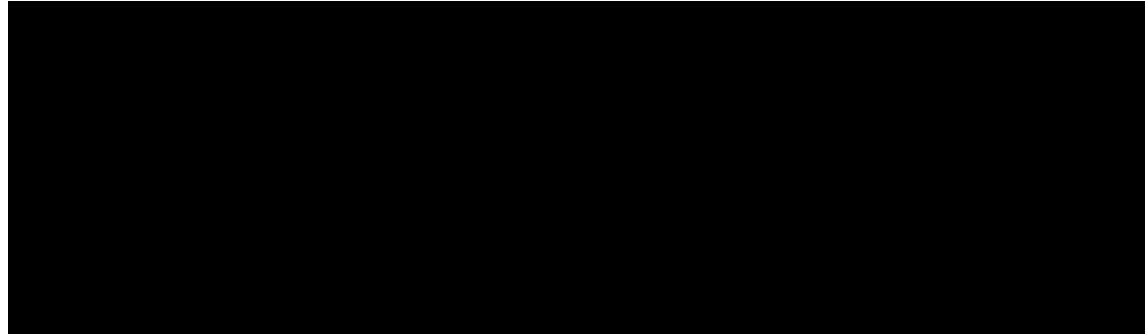


Table 2 Addition volume to prepare Primary culture medium for PMO-22				
	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	124.3 µL of 402.3 µmol/L *1	30.0 µL of 100 µmol/L	15 µL of 100 µmol/L
Primary culture medium	500 µL	375.7 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 3 Addition volume to prepare Primary culture medium for PMO-26

	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	118.6 µL of 421.5 µmol/L *1	30.0 µL of 100 µmol/L	15 µL of 100 µmol/L
Primary culture medium	500 µL	381.4 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 4 Addition volume to prepare Primary culture medium for PMO-R1

	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	121.1 µL of 412.8 µmol/L *1	30.0 µL of 100 µmol/L	15 µL of 100 µmol/L
Primary culture medium	500 µL	378.9 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 5 Addition volume to prepare Primary culture medium for PMO-R2

	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	116.3 µL of 429.9 µmol/L *1	30.0 µL of 100 µmol/L	15 µL of 100 µmol/L
Primary culture medium	500 µL	383.7 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 6 Addition volume to prepare Primary culture medium for PMO-R3

	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	114.7 µL of 436.0 µmol/L *1	30.0 µL of 100 µmol/L	15 µL of 100 µmol/L
Primary culture medium	500 µL	385.3 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

#### 6.4 Culture medium

- Primary culture medium (SkGM™-2)

##### (1) Composition

[REDACTED]

Gentamicin/Amphotericin-B (GAB).

##### (2) Preparation

[REDACTED]

- Differentiation medium (HS-DMEM)

##### (1) Composition

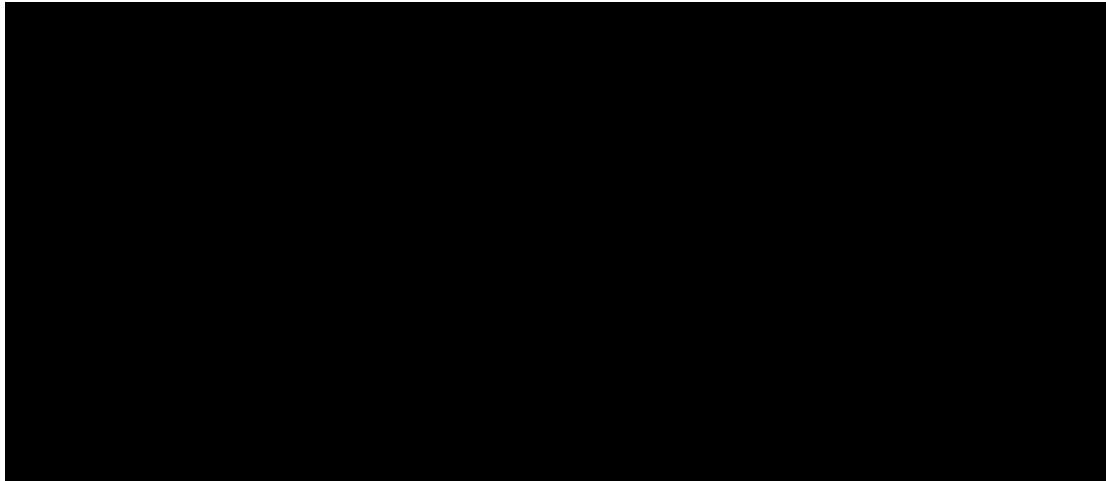
[REDACTED]

##### (2) Preparation

[REDACTED]

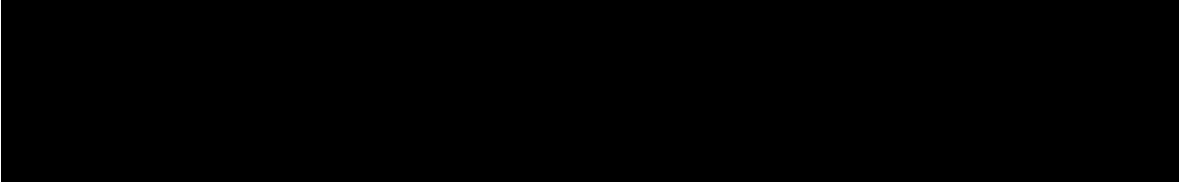
#### 6.5 Cell culture and differentiation

[REDACTED]



6.6 Transfection

6.6.1 Preparation of test substance solution.



6.6.2 Preparation of medium containing test substance.

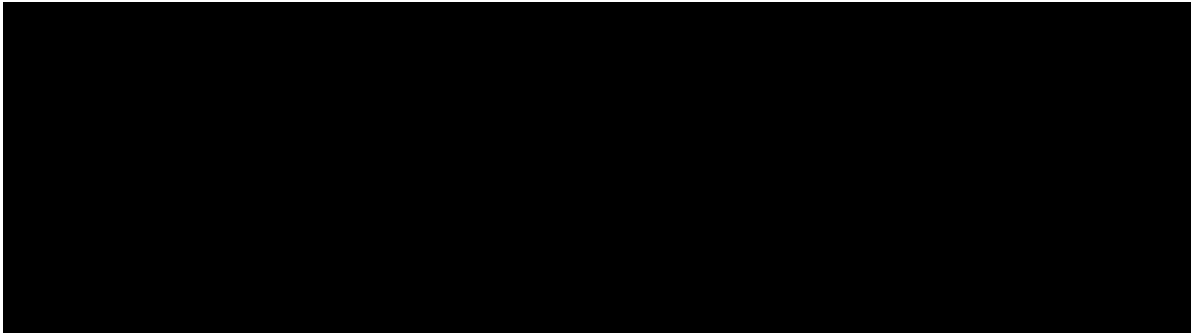


Table 2 Addition volume to prepare Primary culture medium for PMO-22

	0 nmol/L	10 µmol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 µL	124.3 µL of 402.3 µmol/L *1	30.0 µL of 100 µmol/L	15 µL of 100 µmol/L
Primary culture medium	500 µL	375.7 µL	470.0 µL	485.0 µL

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 3 Addition volume to prepare Primary culture medium for PMO-26

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	118.6 $\mu$ L of 421.5 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	381.4 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 4 Addition volume to prepare Primary culture medium for PMO-R1

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	121.1 $\mu$ L of 412.8 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	378.9 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 5 Addition volume to prepare Primary culture medium for PMO-R2

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	116.3 $\mu$ L of 429.9 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	383.7 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 6 Addition volume to prepare Primary culture medium for PMO-R3

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	114.7 $\mu$ L of 436.0 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	385.3 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 7 Addition volume to prepare Primary culture medium for PMO-R4

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	131.6 $\mu$ L of 379.9 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	368.4 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 8 Addition volume to prepare Primary culture medium for PMO-R5

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	126.2 $\mu$ L of 396.3 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	373.8 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 9 Addition volume to prepare Primary culture medium for PMO-R6

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	121.5 $\mu$ L of 411.5 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	378.5 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

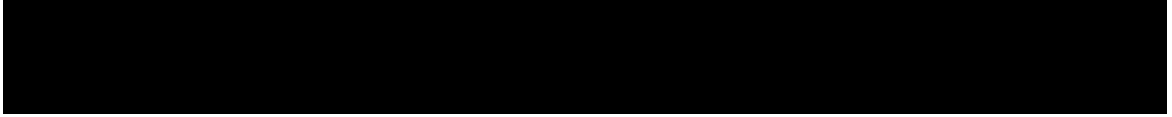
\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

Table 10 Addition volume to prepare Primary culture medium for PMO-R7

	0 nmol/L	10 $\mu$ mol/L as final concentration	600 nmol/L as final concentration	300 nmol/L as final concentration
PMO	0 $\mu$ L	113.5 $\mu$ L of 440.6 $\mu$ mol/L *1	30.0 $\mu$ L of 100 $\mu$ mol/L	15 $\mu$ L of 100 $\mu$ mol/L
Primary culture medium	500 $\mu$ L	386.5 $\mu$ L	470.0 $\mu$ L	485.0 $\mu$ L

\*1: This volume was calculated by the actual concentration measured by NanoDrop One.

6.6.3 Preparation of Endo-Porter in Primary culture medium



6.6.4 Exposure

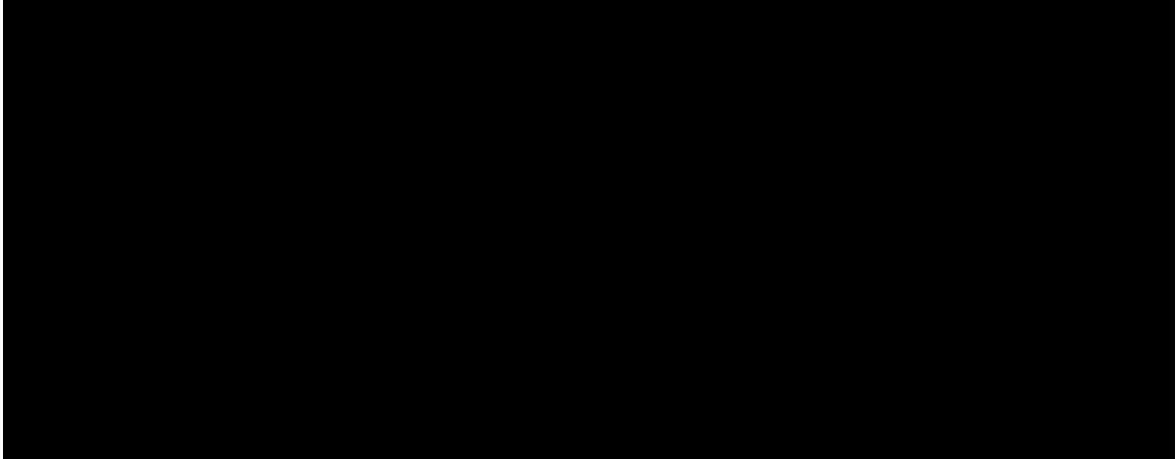
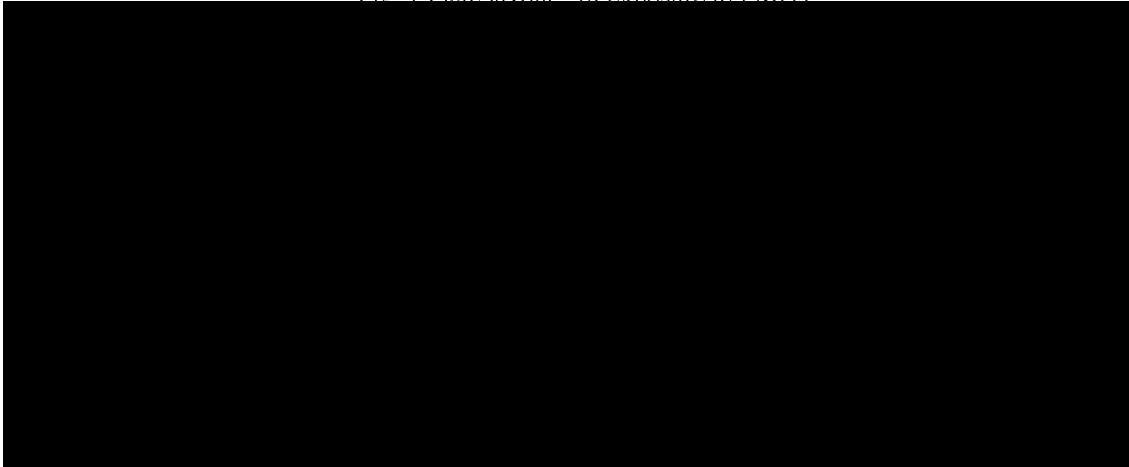


Fig. 1 Plate layout\*1 of exposure to PMOs

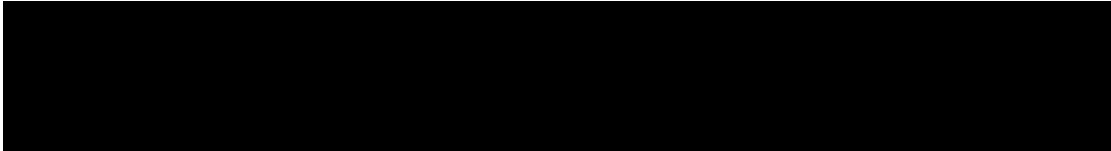


\*2: There was no cells in wells and only 500  $\mu$ L of fresh Primary culture medium without Endo-Porter DMSO was added at the time of transfection

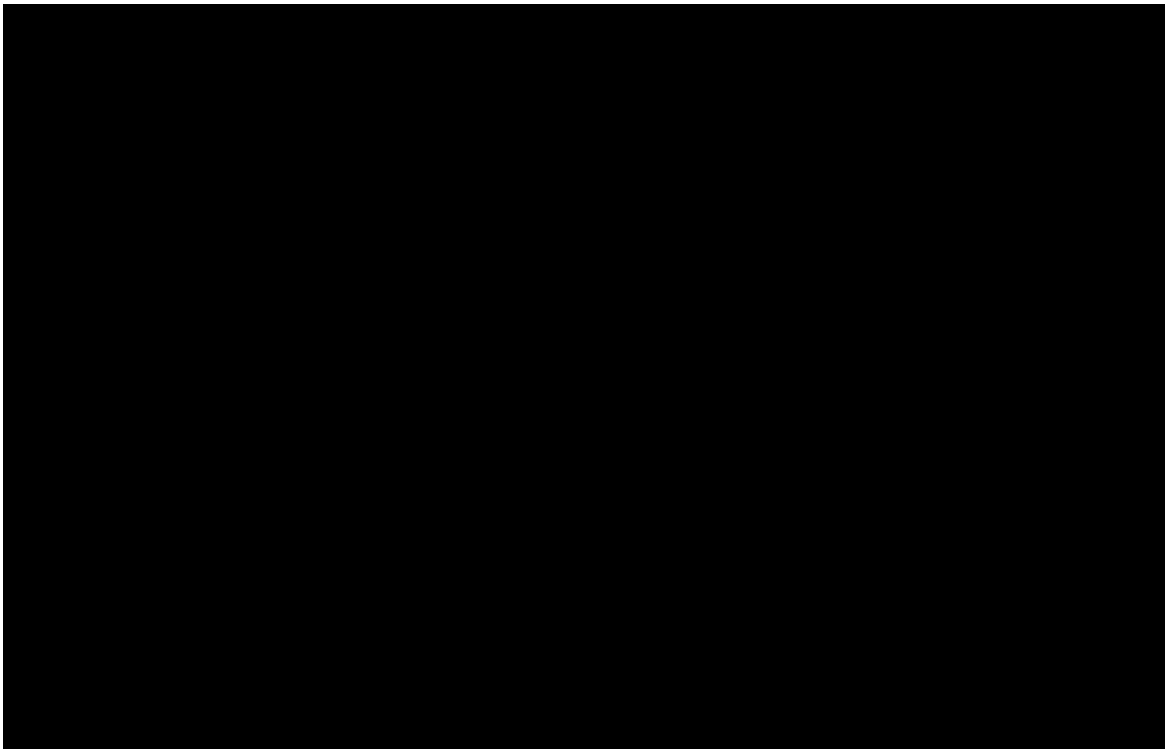
#### 6.7 Gene Expression analysis



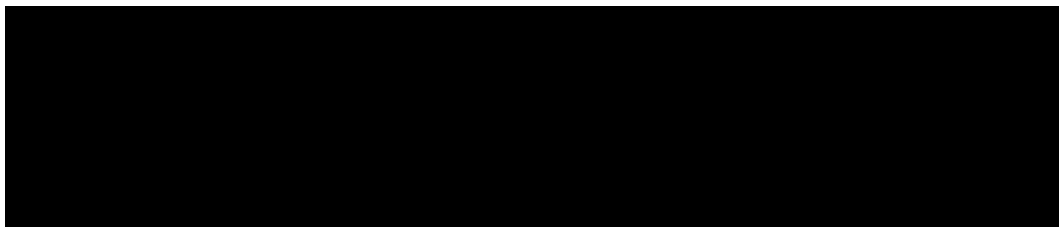
##### 6.7.2 RNA quality analysis by the 4200 TapeStation



##### 6.7.3 cDNA synthesis



##### 6.7.4 Electrophoresis







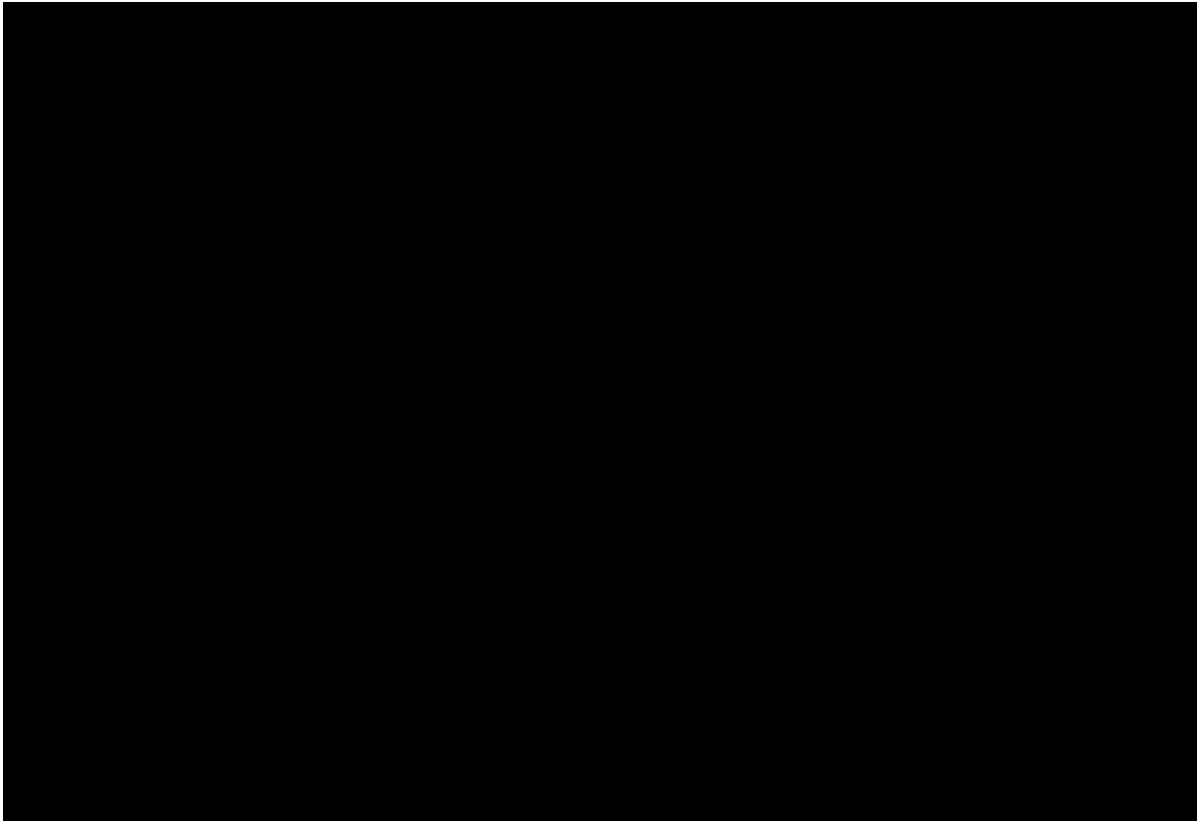
[REDACTED]

[REDACTED]  
[REDACTED]  
[REDACTED]  
[REDACTED]  
[REDACTED]

[REDACTED]			
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]			
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[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

[REDACTED]

7.2 Assessment of skipping efficiency of PMOs



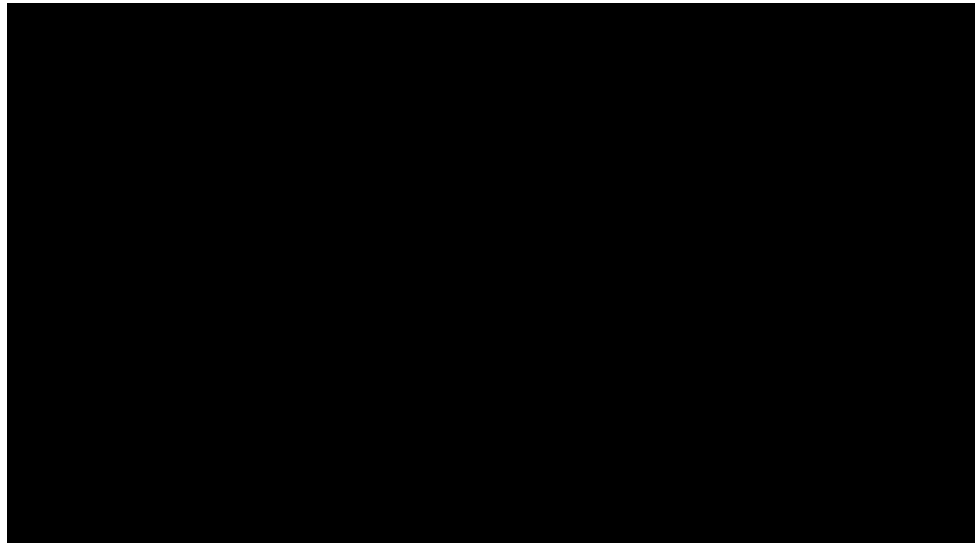


Fig. 2 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-22 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-22 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-22 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-22 at 600 nmol/L (replicate 1, 2 and 3, respectively)

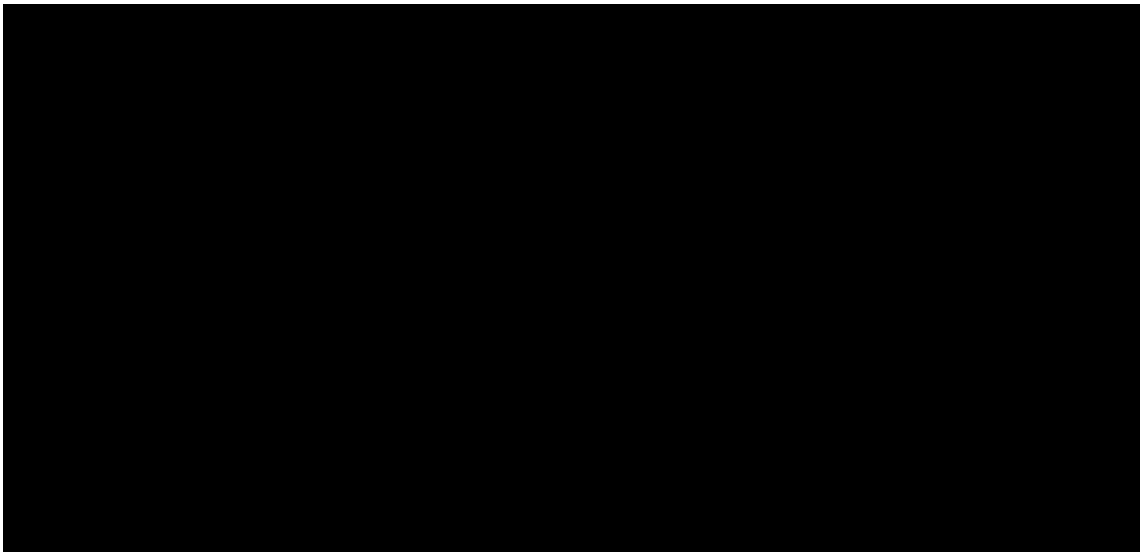


Fig. 3 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-22 exposed cells (Dilution factor 20, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-22 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-22 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-22 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-22 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

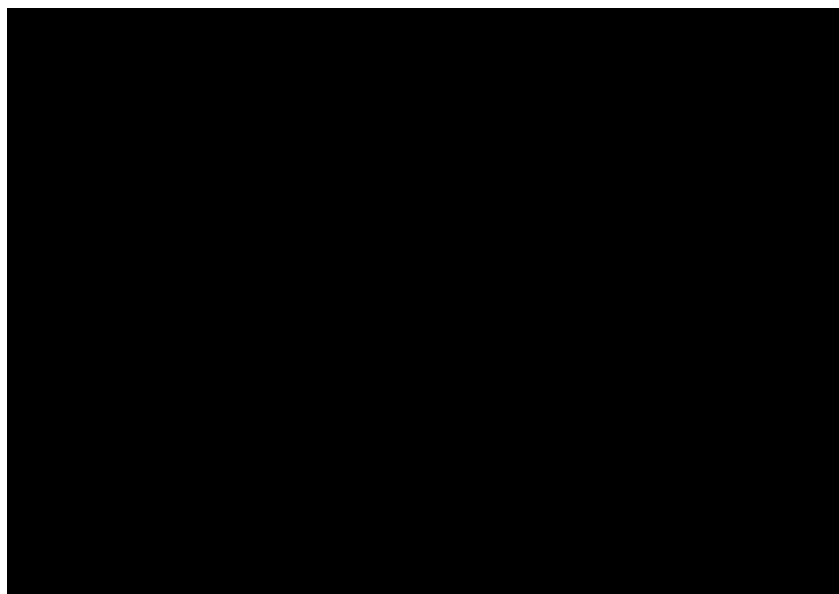


Fig. 4 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-26 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-26 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-26 at 300 nmol/L (replicate 1, 2 and 3, respectively)

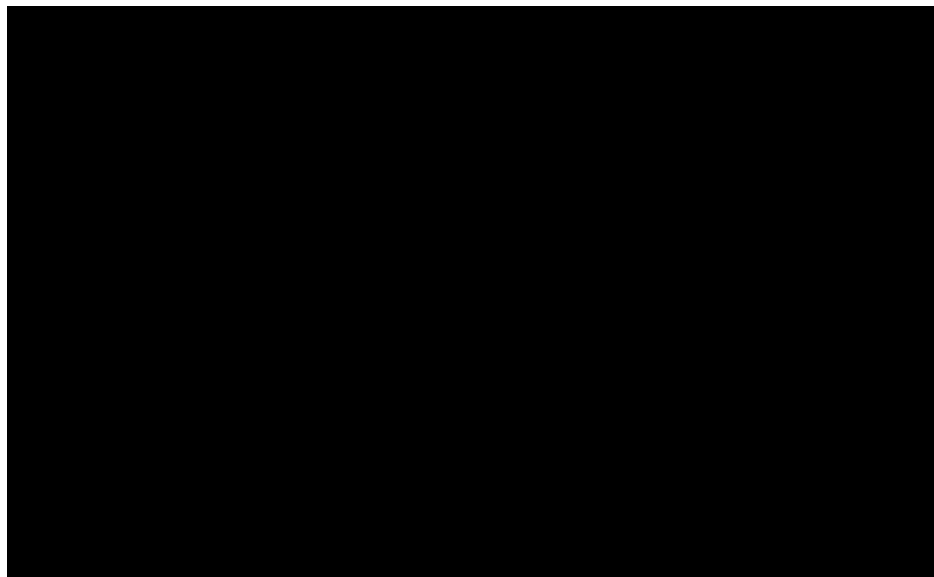


Fig. 5 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-26 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-26 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-26 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

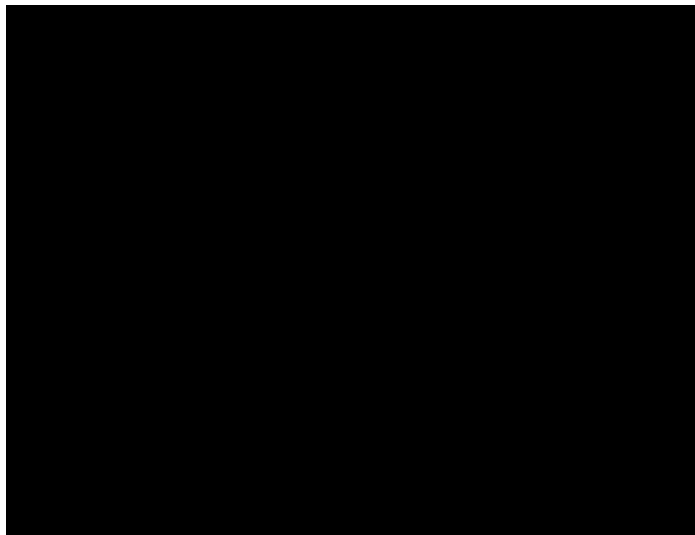


Fig. 6 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-26 exposed cells (Dilution factor 20, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-26 (replicate 1, 2 and 3, respectively)

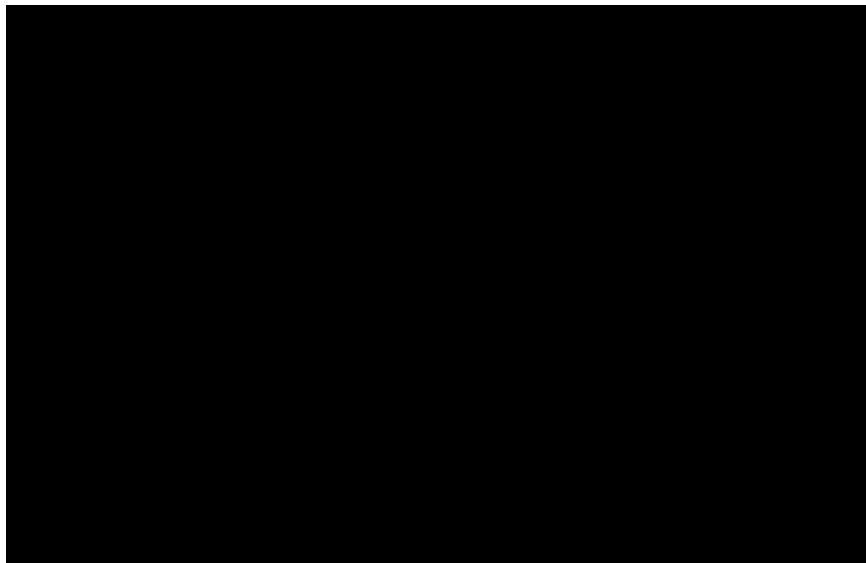


Fig. 7 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-26 exposed cells (Dilution factor 20, approximately 365 bp fragment)  
A1: Ladder Marker, B1 and C1: PMO-26 at 300 nmol/L (replicate 1 and 3, respectively), D1, E1 and F1: PMO-26 at 600 nmol/L (replicate 1, 2 and 3, respectively), G1 and H1: PMO-26 at 10  $\mu$ mol/L (replicate 2 and 3, respectively)

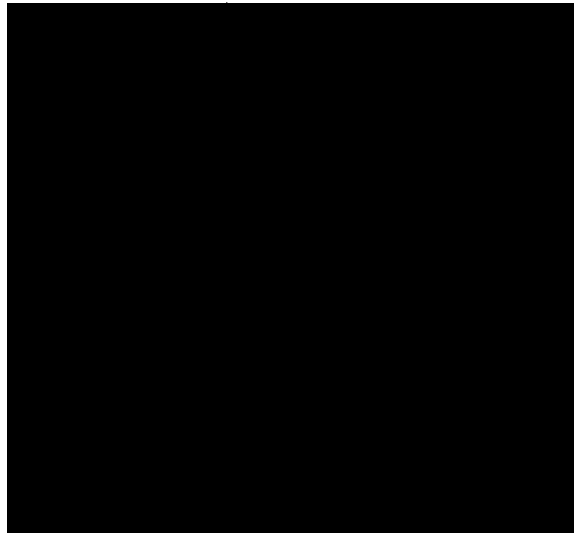


Fig. 8 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-26 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1: PMO-26 at 300 nmol/L (replicate 2), C1: PMO-26 at 10  $\mu$ mol/L (replicate 1)

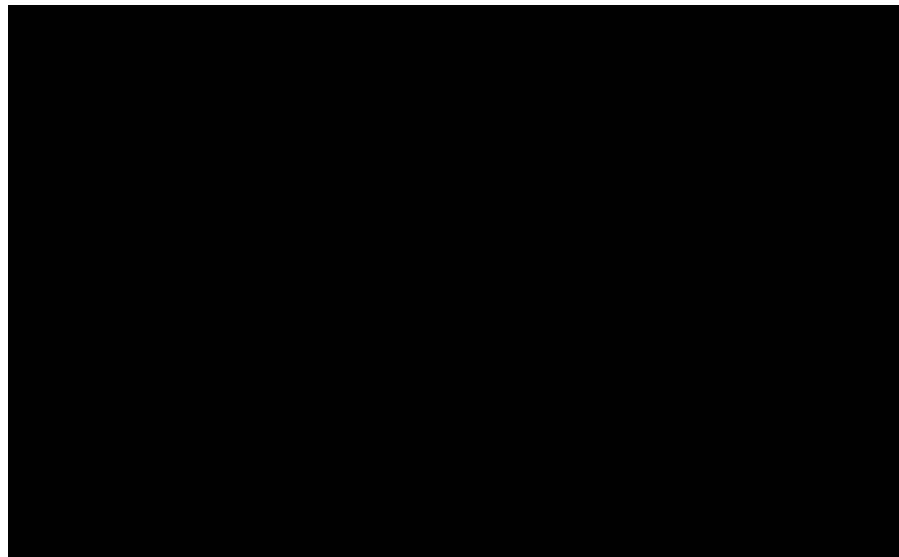


Fig. 9 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R1 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-R1 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R1 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-R1 at 600 nmol/L (replicate 1 and 2, respectively)

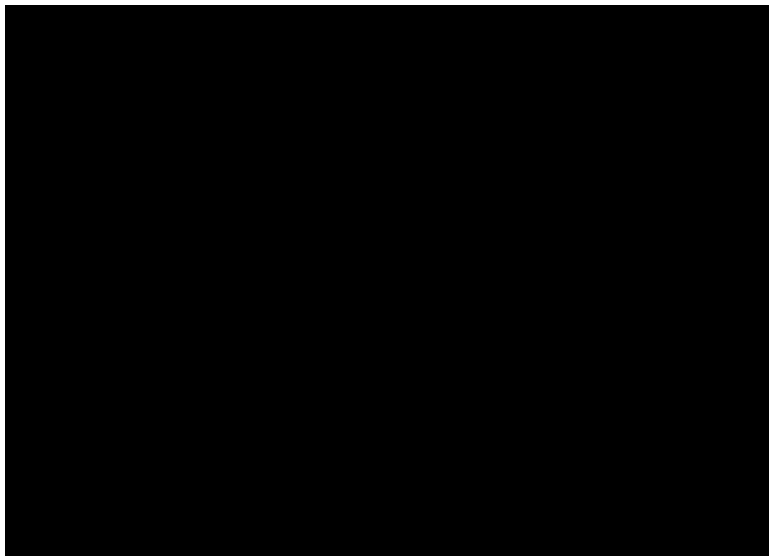


Fig. 10 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R1 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1: PMO-R1 at 600 nmol/L (replicate 3), C1, D1 and E1: PMO-R1 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

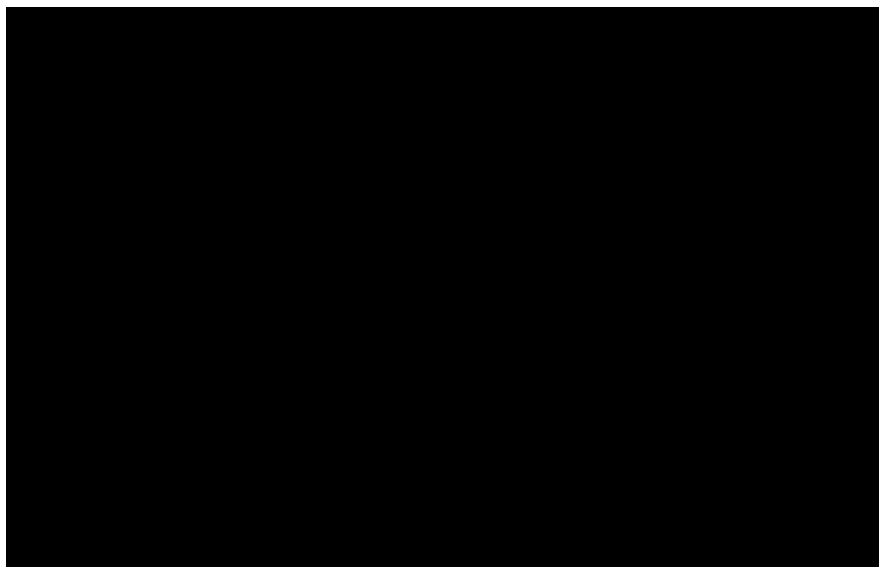


Fig. 11 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R1 exposed cells (Dilution factor 20, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R1 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R1 at 300 nmol/L (replicate 1, 2 and 3, respectively)



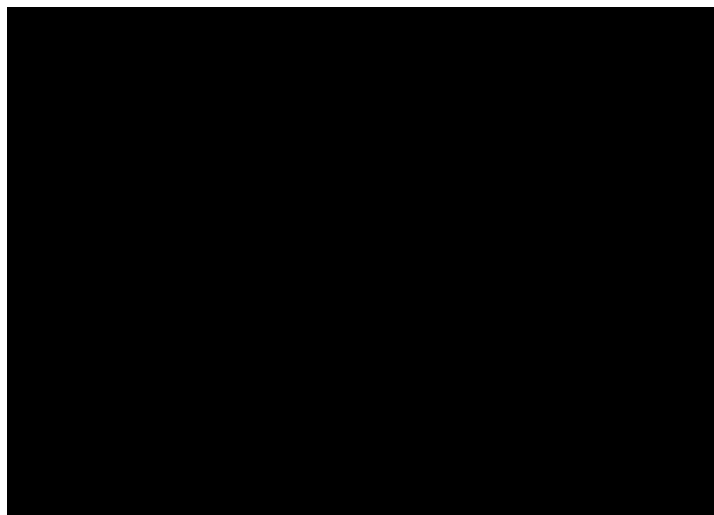


Fig. 12 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R1 exposed cells (Dilution factor 20, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-R1 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1 and F1: PMO-R1 at 10  $\mu$ mol/L (replicate 1 and 3, respectively)

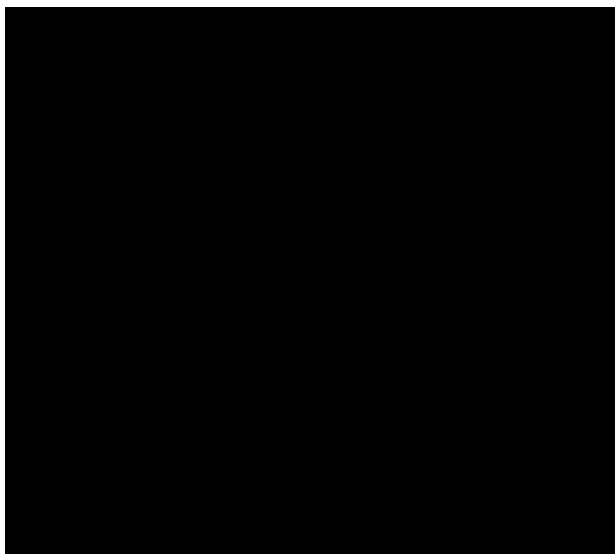


Fig. 13 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R1 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-R1 at 10  $\mu$ mol/L (replicate 2)

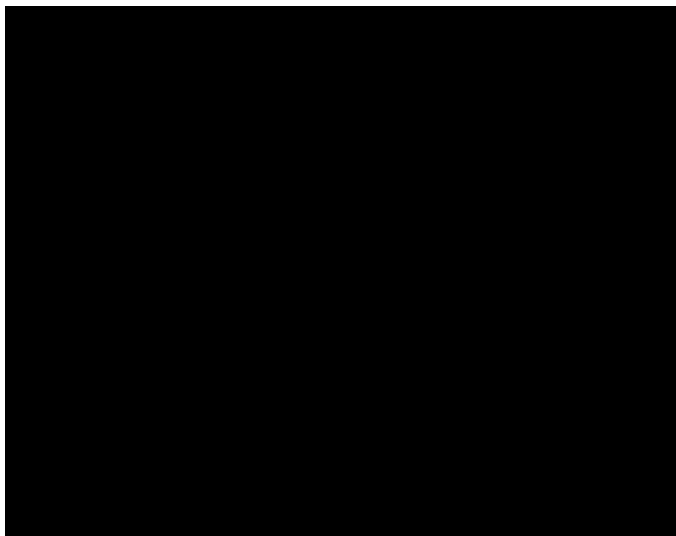


Fig. 14 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R2 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-R2 (replicate 1, 2 and 3, respectively)

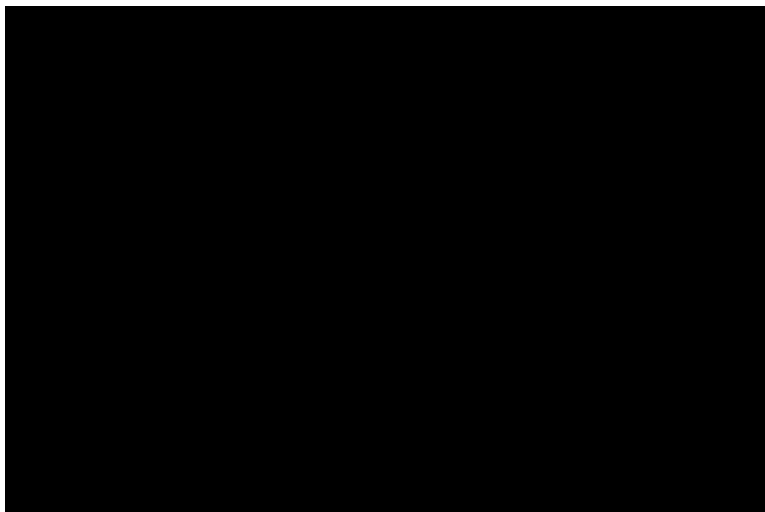


Fig. 15 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R2 exposed cells (Dilution factor 1, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-R2 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R2 at 600 nmol/L (replicate 1, 2 and 3, respectively)

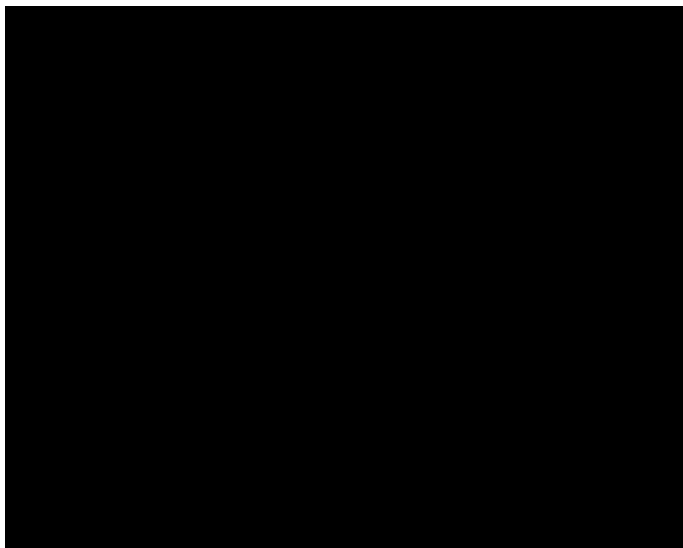


Fig. 16 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R2 exposed cells (Dilution factor 5, approximately 153 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-R2 at 10  $\mu\text{mol/L}$  (replicate 1, 2 and 3, respectively)

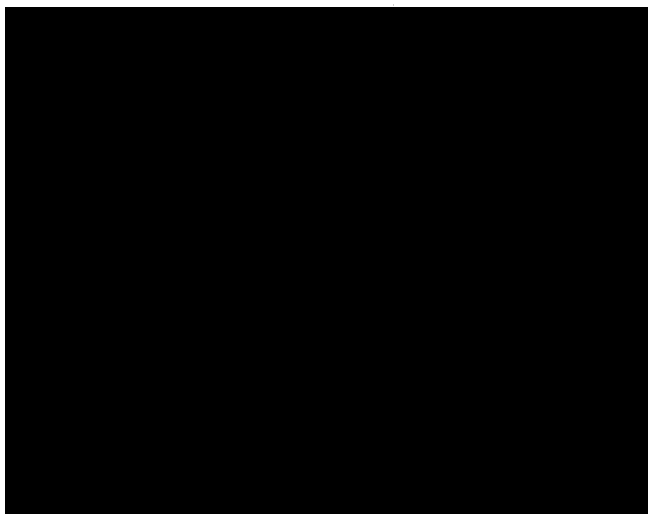


Fig. 17 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R2 exposed cells (Dilution factor 20, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-R2 (replicate 1, 2 and 3, respectively)

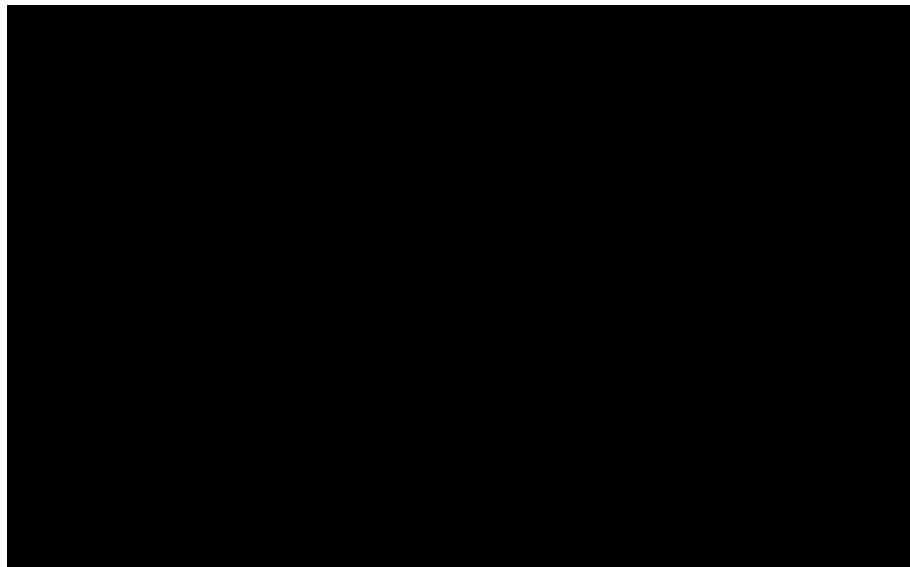


Fig. 18 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R2 exposed cells (Dilution factor 20, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-R2 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R2 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-R2 at 10  $\mu$ mol/L (replicate 1 and 2, respectively)

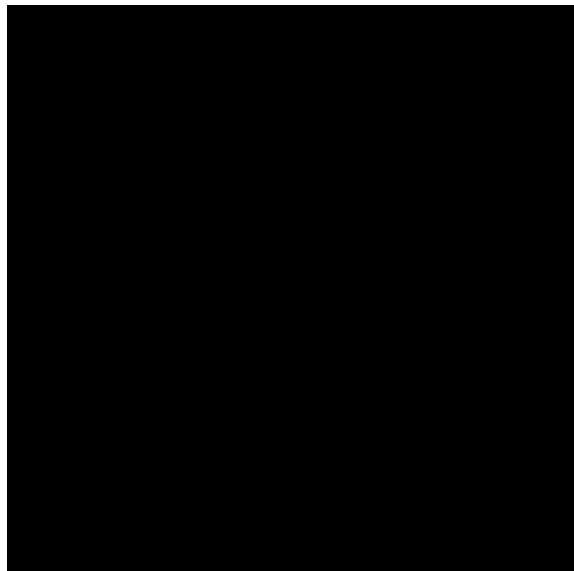


Fig. 19 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R2 exposed cells (Dilution factor 20, approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-R2 at 10  $\mu$ mol/L (replicate 3)

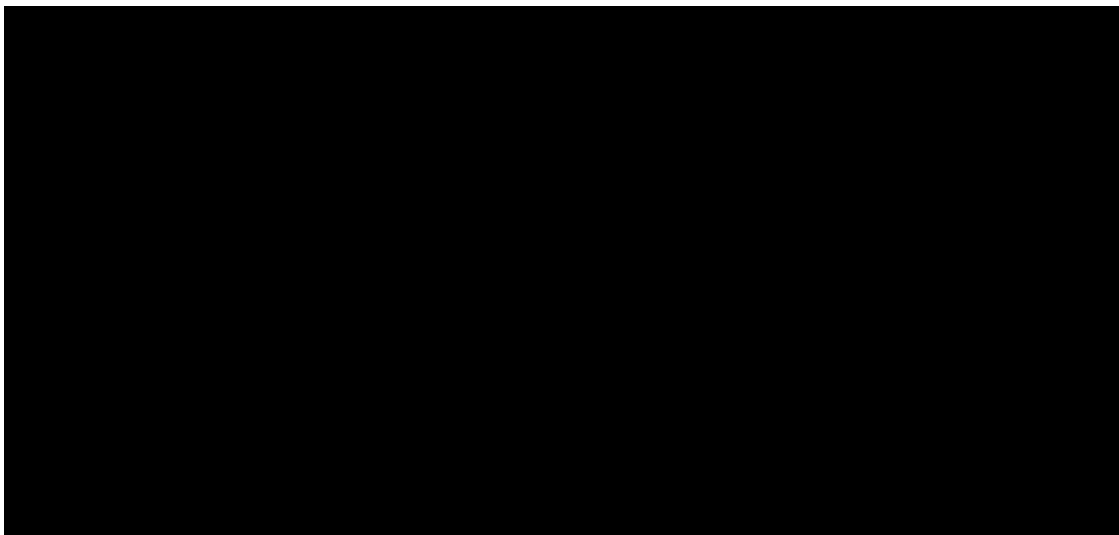


Fig. 20 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R3 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R3 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R3 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-R3 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-R3 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

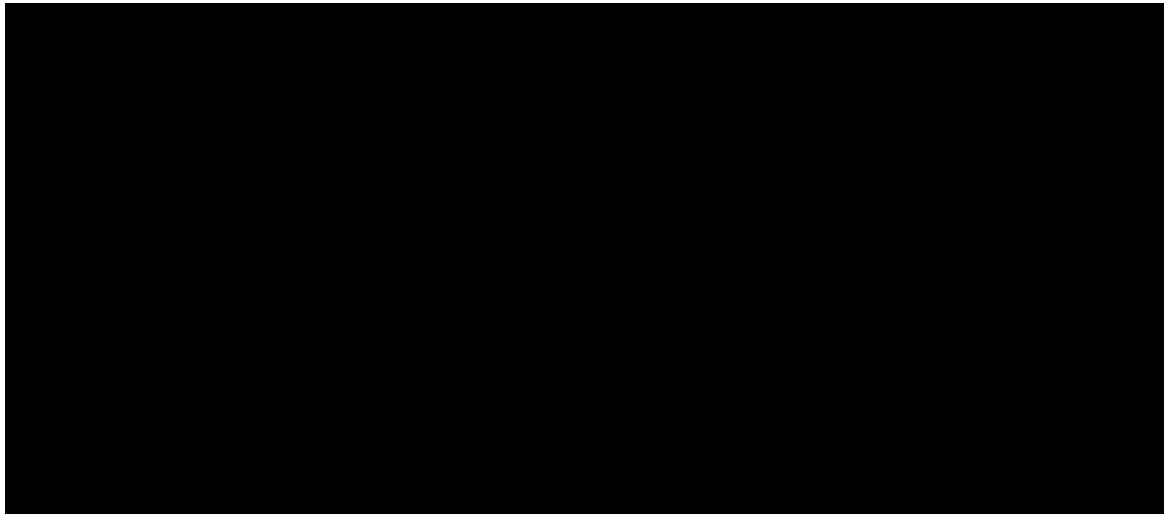


Fig. 21 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R3 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R3 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R3 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-R3 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-R3 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

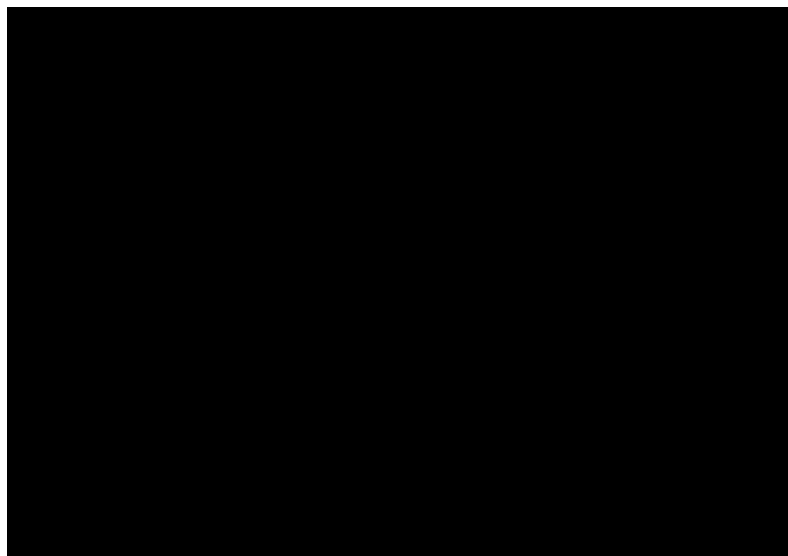


Fig. 22 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R4 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R4 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R4 at 300 nmol/L (replicate 1, 2 and 3, respectively)

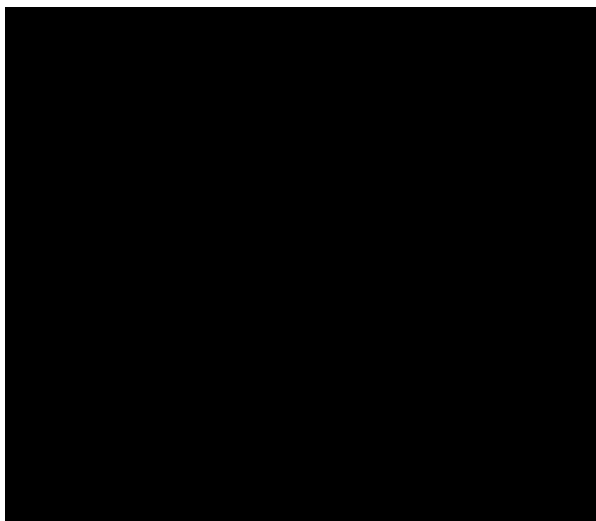


Fig. 23 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R4 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-R4 at 600 nmol/L (replicate 1, 2 and 3, respectively)

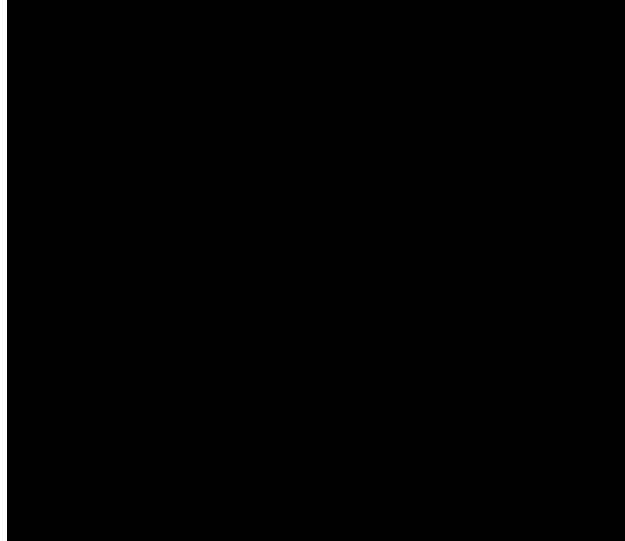


Fig. 24 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R4 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: control of PMO-R4 (replicate 1, 2 and 3, respectively)

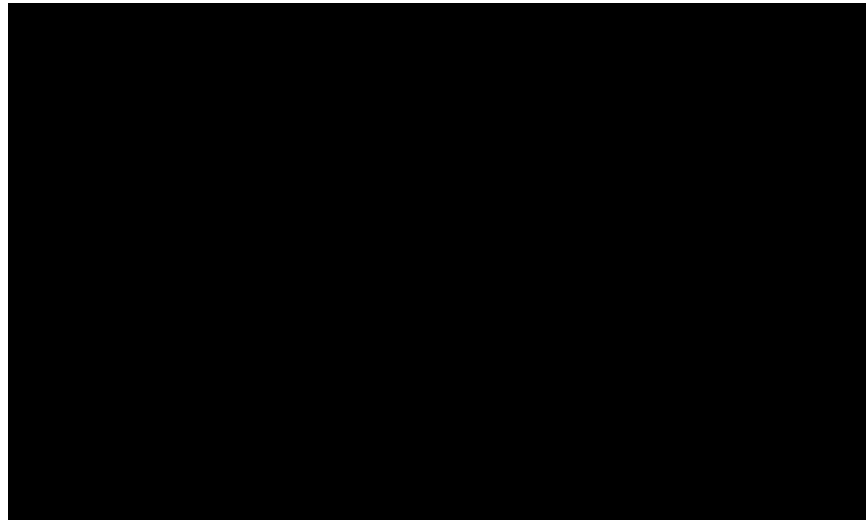


Fig. 25 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R4 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1, C1 and D1: PMO-R4 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R4 at 600 nmol/L (replicate 1, 2 and 3, respectively)

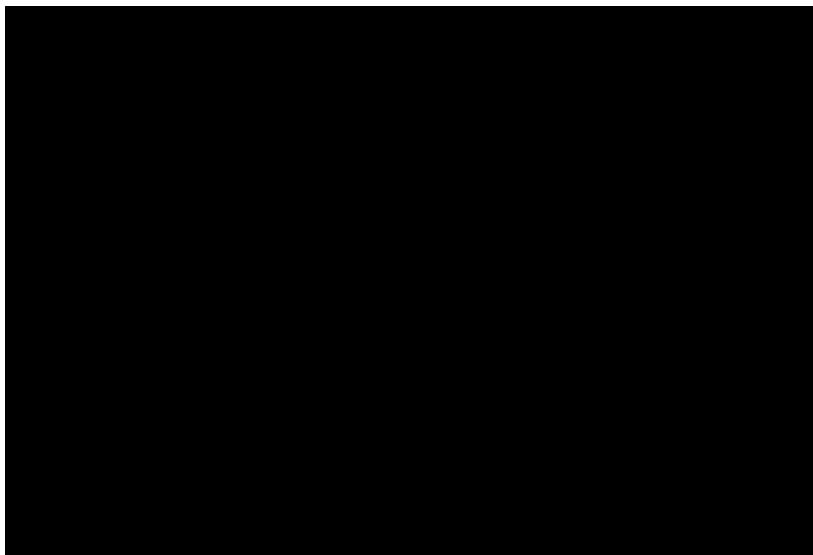


Fig. 26 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R4 exposed cells (Dilution factor 40, approximately 365 and 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-R4 at 10  $\mu\text{mol/L}$  (replicate 1, 2 and 3, respectively)

Exclamation marks indicate that a noise peak was detected outside upper or lower marker.

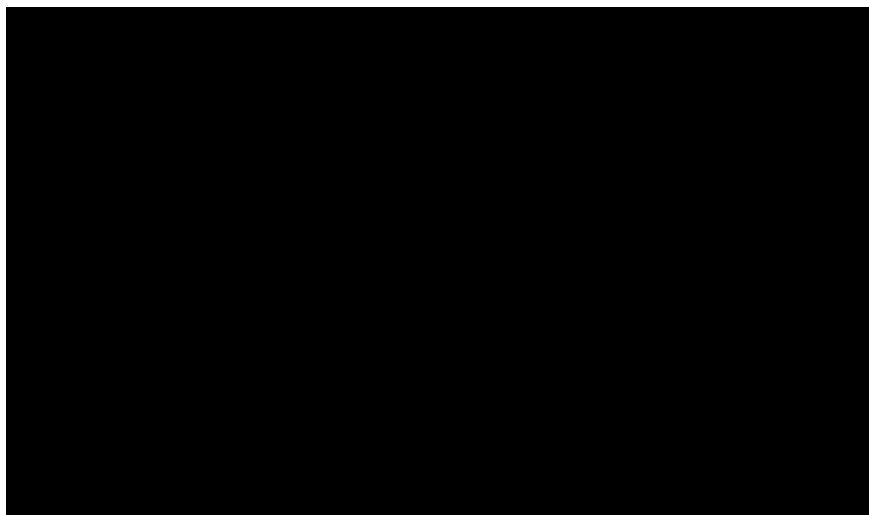


Fig. 27 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R5 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R5 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R5 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-R5 at 600 nmol/L (replicate 1 and 2, respectively)





Fig. 28 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R5 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1: PMO-R5 at 600 nmol/L (replicate 3), C1, D1 and E1: PMO-R5 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

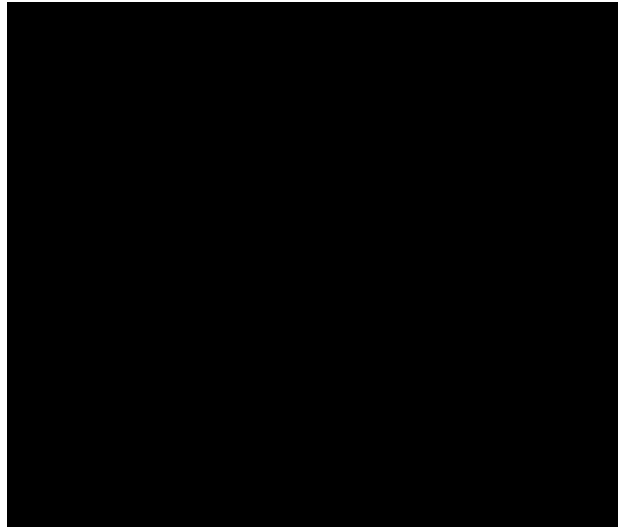


Fig. 29 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R5 exposed cells (Dilution factor 60, approximately 365 bp fragment)

A1: Ladder Marker, B1: control of PMO-R5 (replicate 1), C1: PMO-R5 at 300 nmol/L (replicate 3)

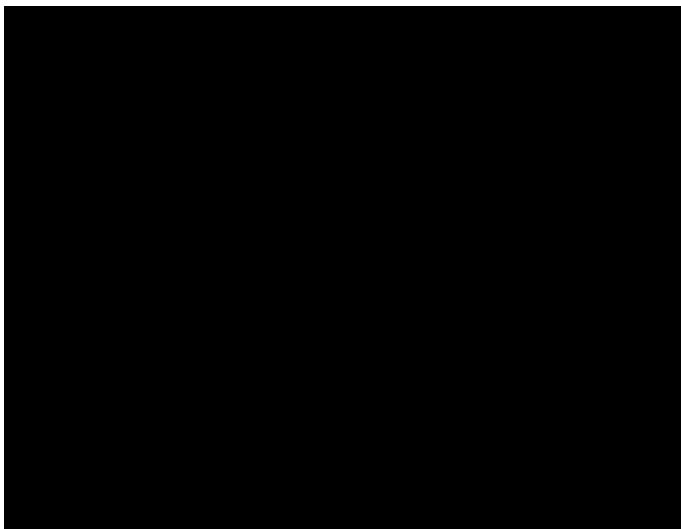


Fig. 30 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R5 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1 and C1: control of PMO-R5 (replicate 2 and 3, respectively), D1 and E1: PMO-R5 at 300 nmol/L (replicate 1 and 2, respectively)

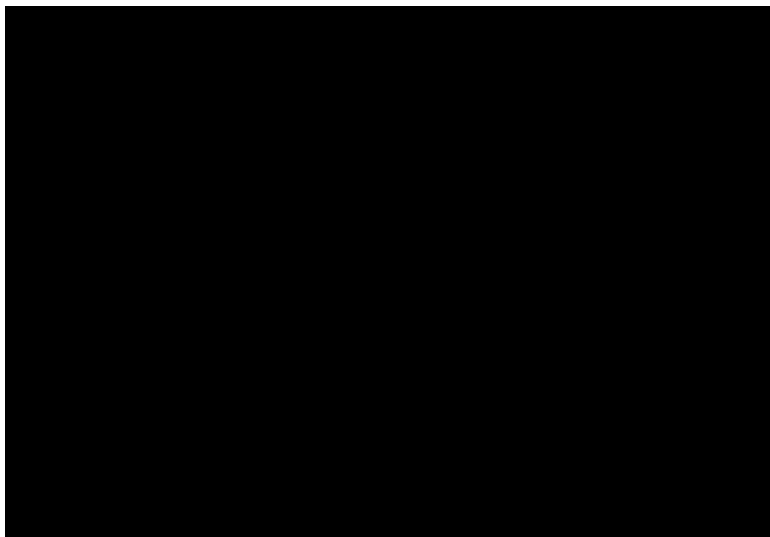


Fig. 31 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R5 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-R5 at 600 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R5 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

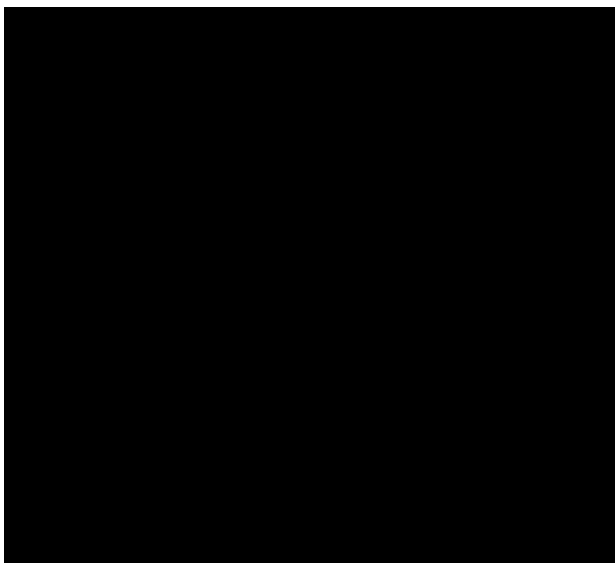


Fig. 32 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R6 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R6 (replicate 1, 2 and 3, respectively)

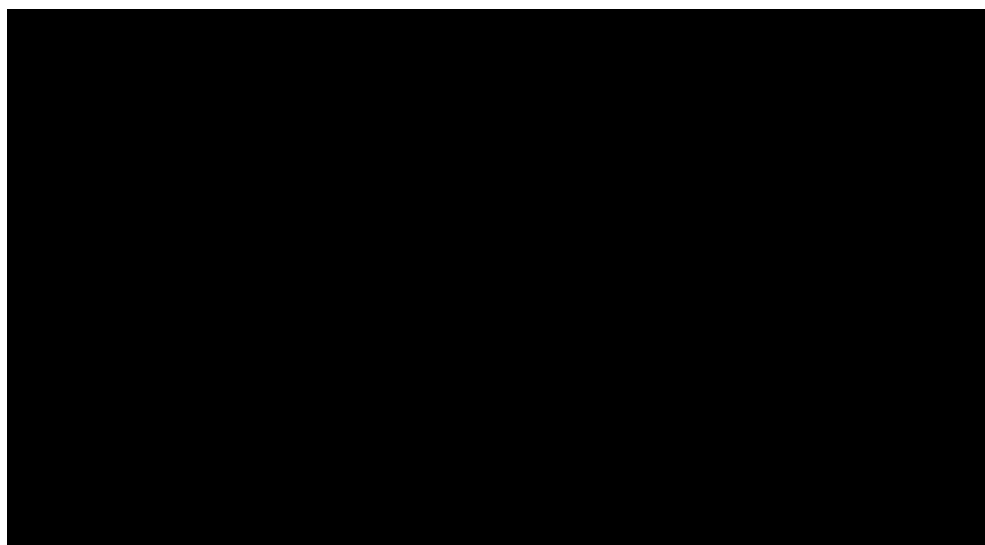


Fig. 33 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R6 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-R6 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R6 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-R6 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

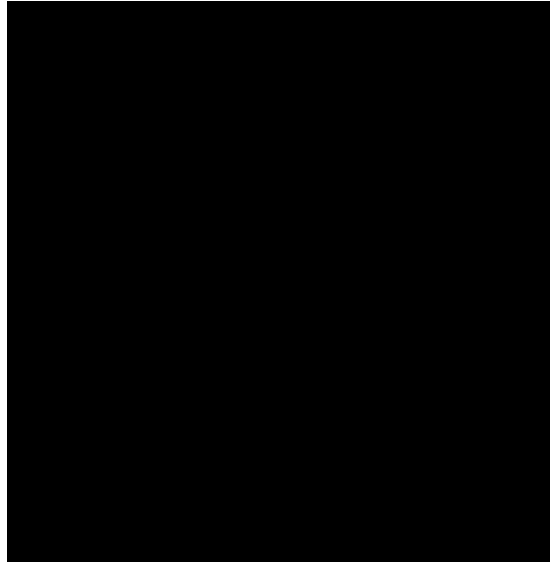


Fig. 34 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R6 exposed cells (Dilution factor 60, approximately 365 bp fragment)  
A1: Ladder Marker, B1: control of PMO-R6 (replicate 1)



Fig. 35 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R6 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1 and C1: control of PMO-R6 (replicate 2 and 3, respectively)

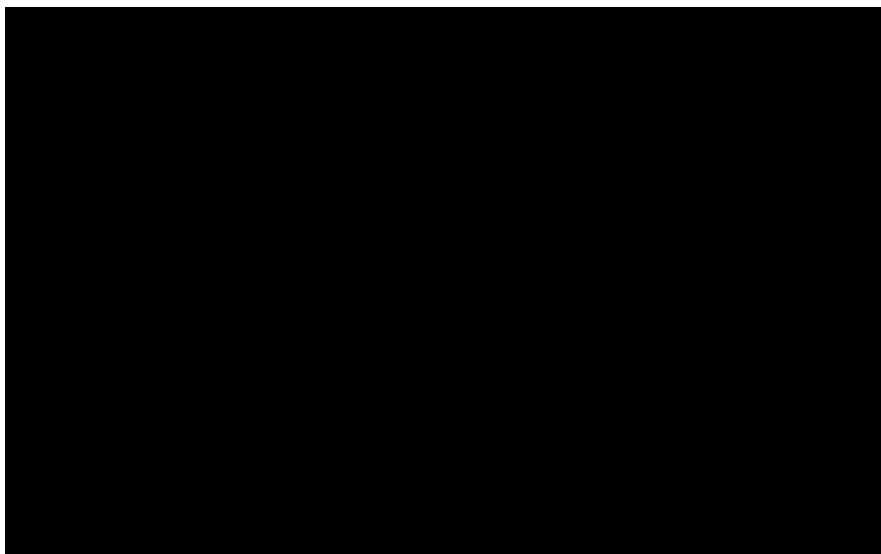


Fig. 36 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R6 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: PMO-R6 at 300 nmol/L (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R6 at 600 nmol/L (replicate 1, 2 and 3, respectively), H1 and A2: PMO-R6 at 10  $\mu$ mol/L (replicate 1 and 2, respectively)



Fig. 37 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R6 exposed cells (Dilution factor 40, approximately 365 bp fragment)

A1: Ladder Marker, B1: PMO-R6 at 10  $\mu$ mol/L (replicate 3)

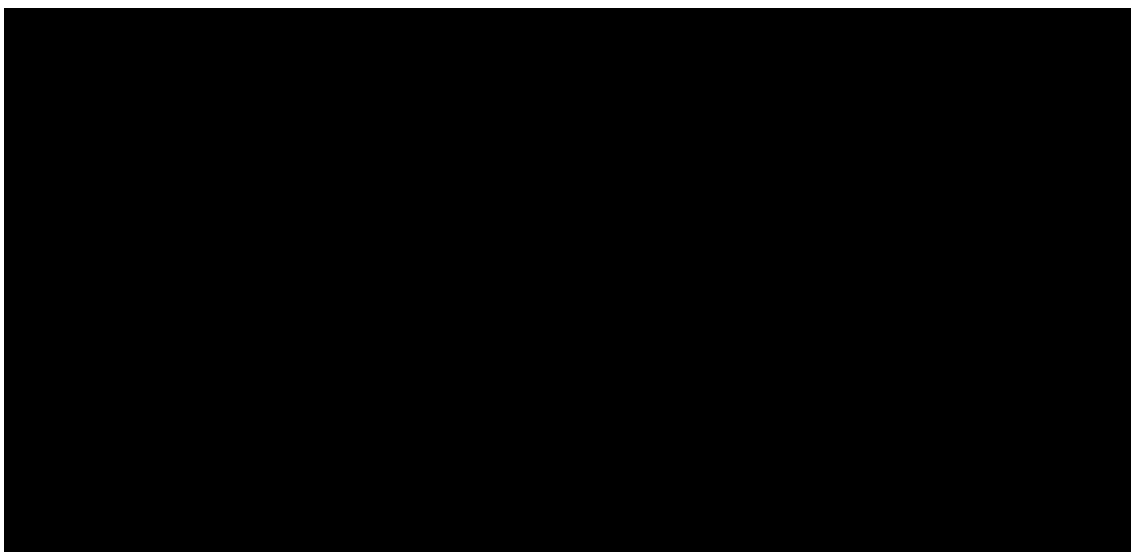


Fig. 38 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R7 exposed cells (Dilution factor 1, approximately 153 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R7 (replicate 1, 2 and 3, respectively), E1, F1 and G1: PMO-R7 at 300 nmol/L (replicate 1, 2 and 3, respectively), H1, A2 and B2: PMO-R7 at 600 nmol/L (replicate 1, 2 and 3, respectively), C2, D2 and E2: PMO-R7 at 10  $\mu$ mol/L (replicate 1, 2 and 3, respectively)

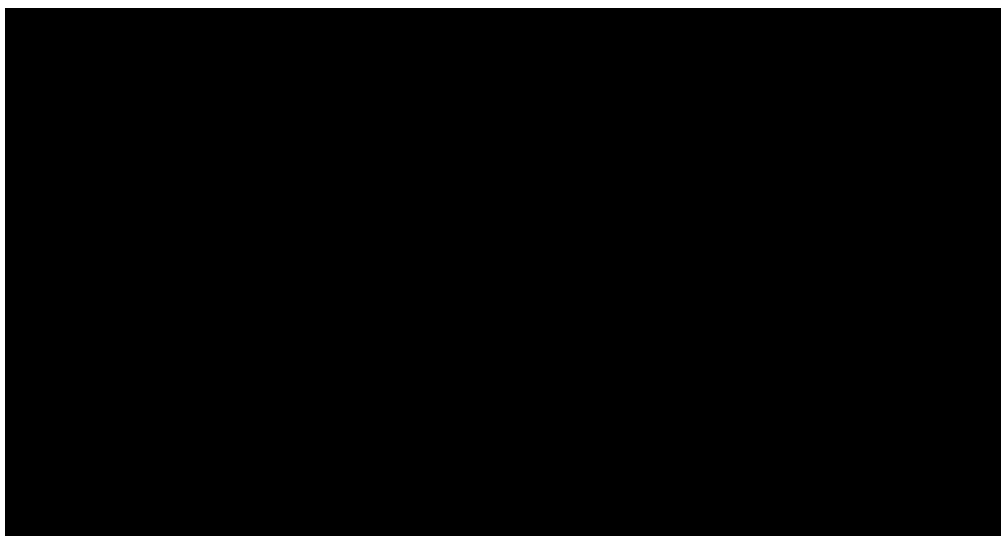


Fig. 39 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R7 exposed cells (Dilution factor 30, approximately 365 bp fragment)

A1: Ladder Marker, B1, C1 and D1: control of PMO-R7 (replicate 1, 2 and 3, respectively), E1 and F1: PMO-R7 at 300 nmol/L (replicate 1 and 3, respectively), G1, H1 and A2: PMO-R7 at 600 nmol/L (replicate 1, 2 and 3, respectively), B2 and C2: PMO-R7 at 10  $\mu$ mol/L (replicate 1 and 2, respectively)

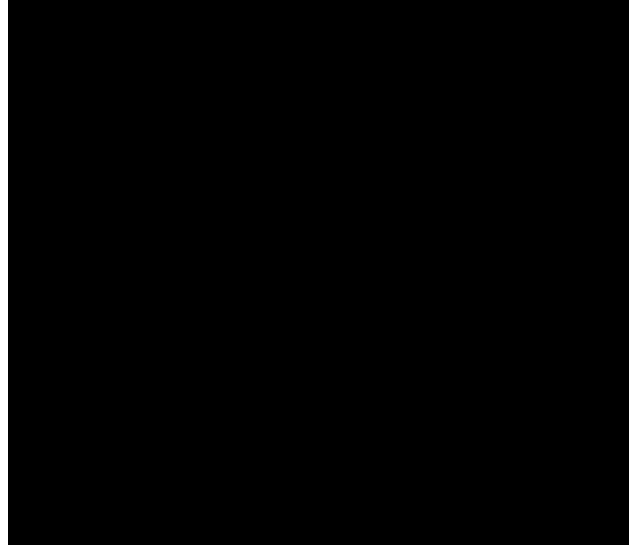
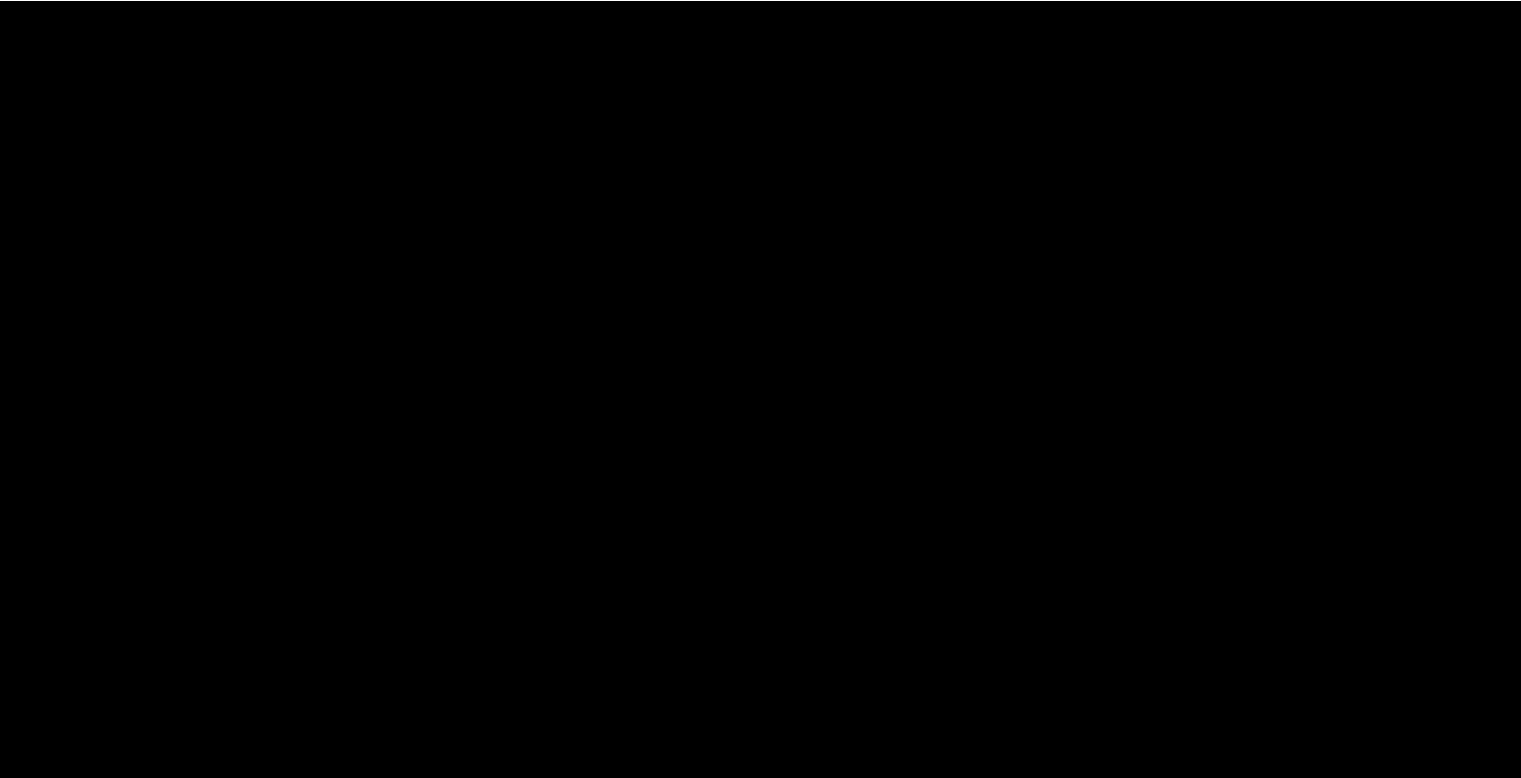
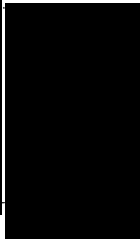


Fig. 40 Electrophoresis by 4200TapeStation for amplified DMD gene of PMO-R7 exposed cells (Dilution factor 40, approximately 365 bp fragment)  
A1: Ladder Marker, B1: PMO-R7 at 300 nmol/L (replicate 2), C1: PMO-R7 at 10  $\mu$ mol/L (replicate 3)

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Table 12 Exon skipping efficiency by 4200TapeStation (PMO-22 exposed cells)

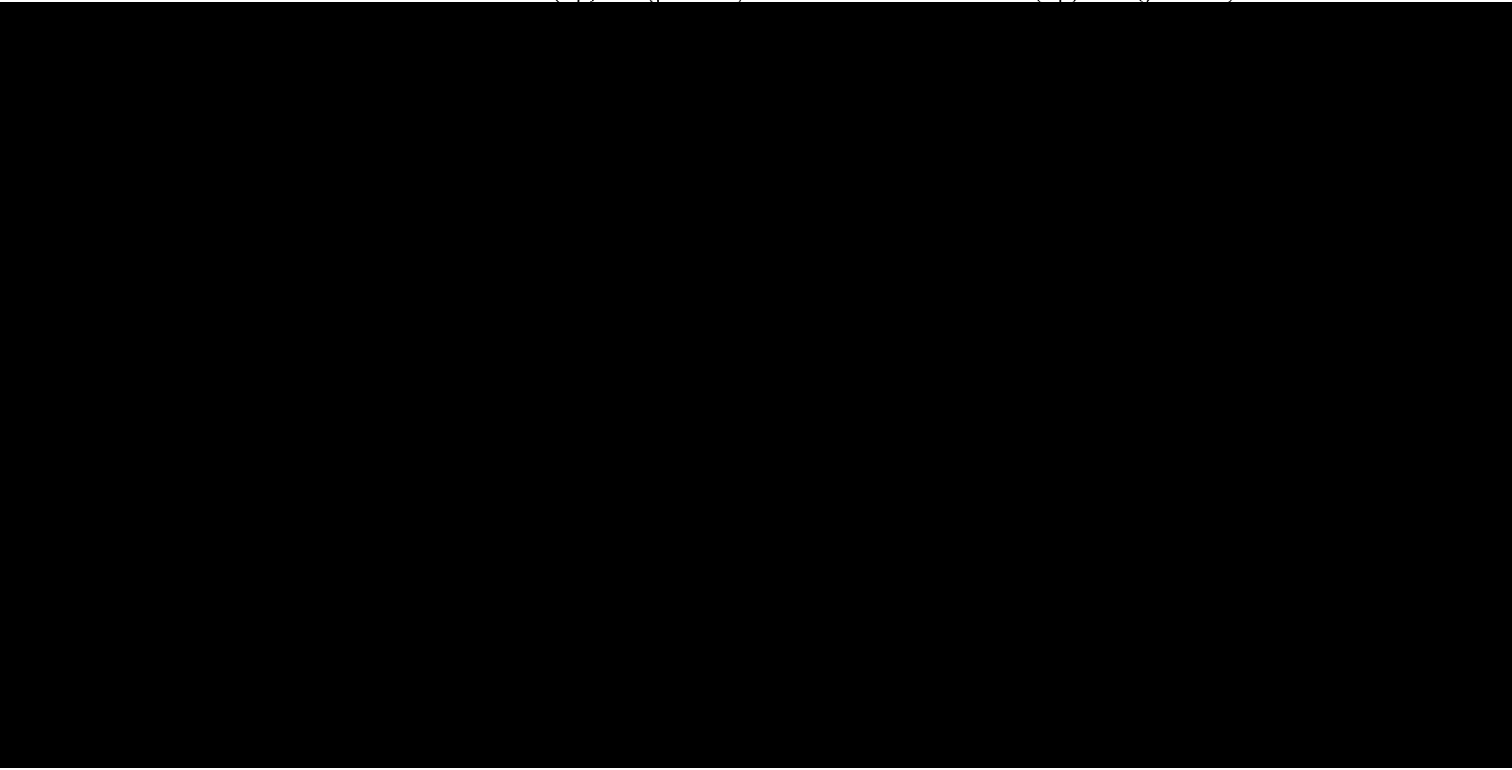
Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable



936-22-M-0661

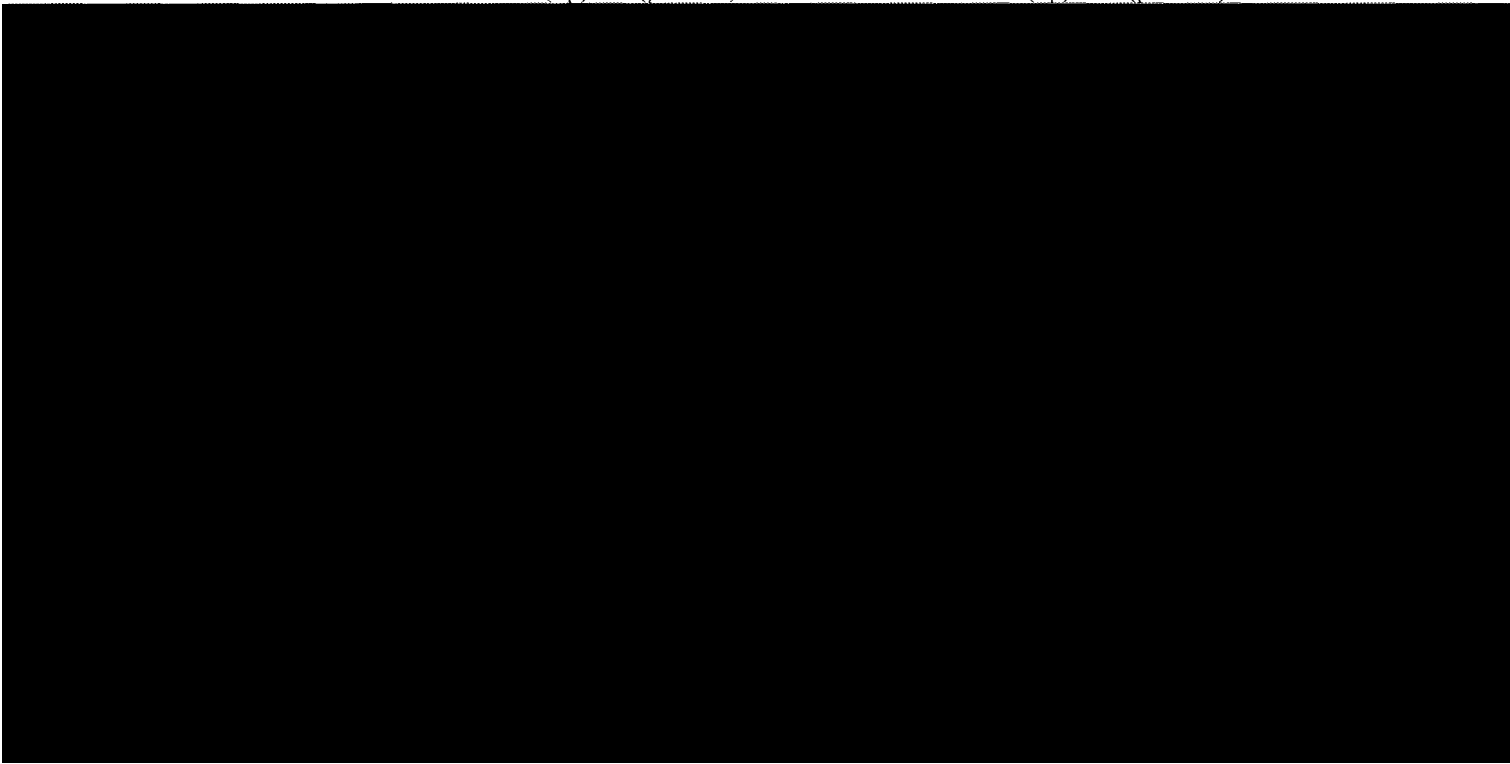
Table 13 Exon skipping efficiency by 4200TapeStation (PMO-26 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-22-M-0661



Table 14 Exon skipping efficiency by 4200TapeStation (PMO-R1 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-22-M-0661

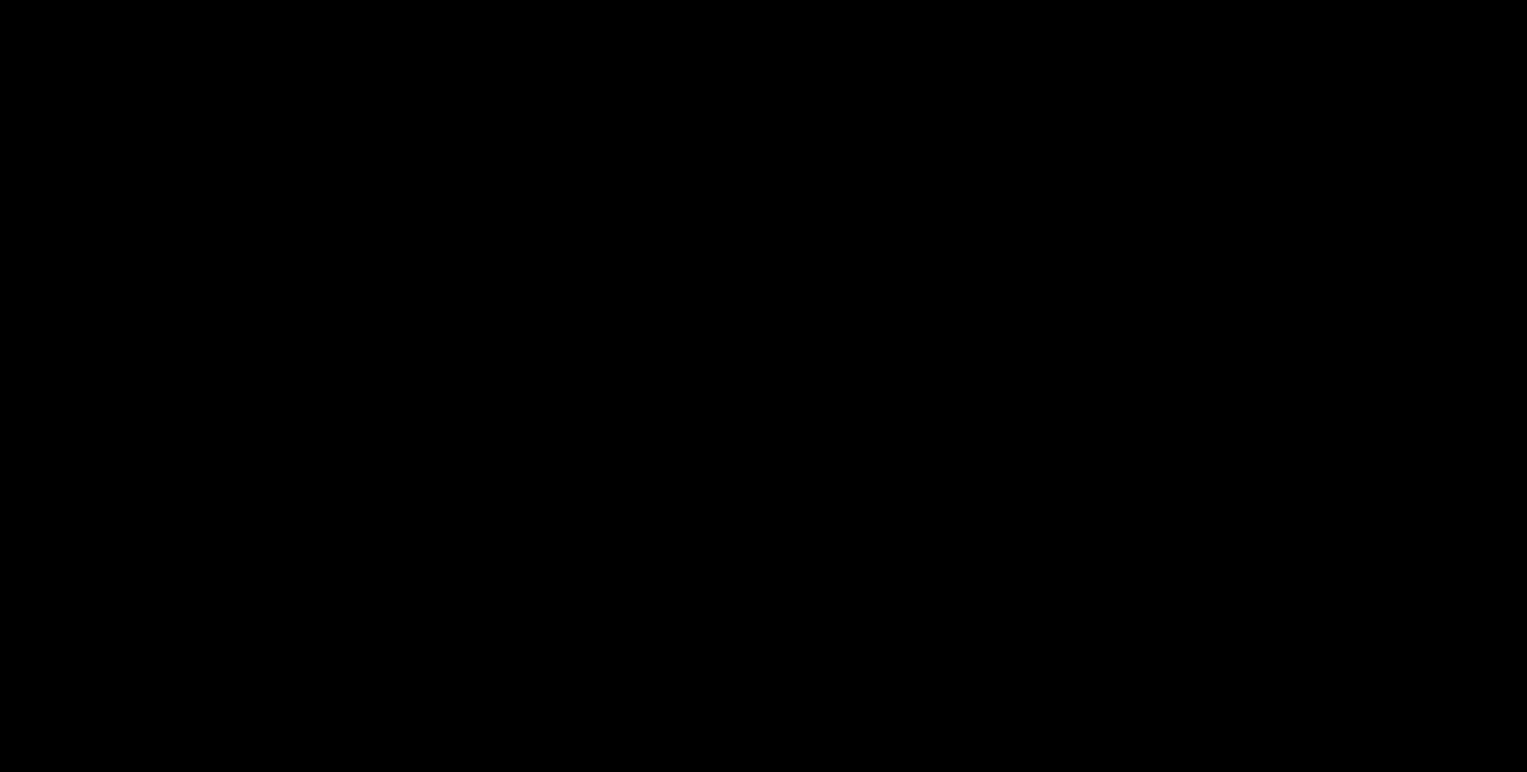
Table 15 Exon skipping efficiency by 4200TapeStation (PMO-R2 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-22-M-0661

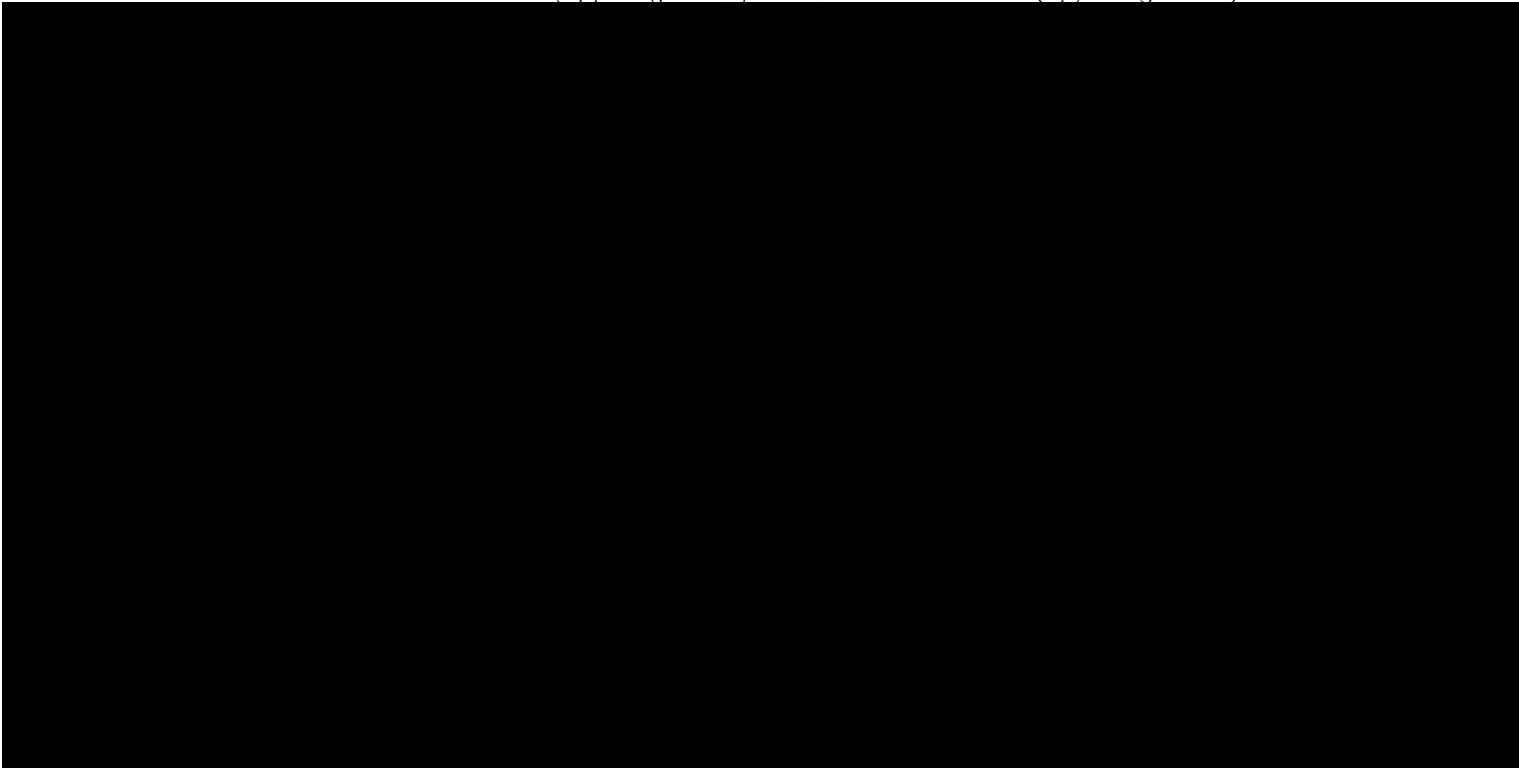
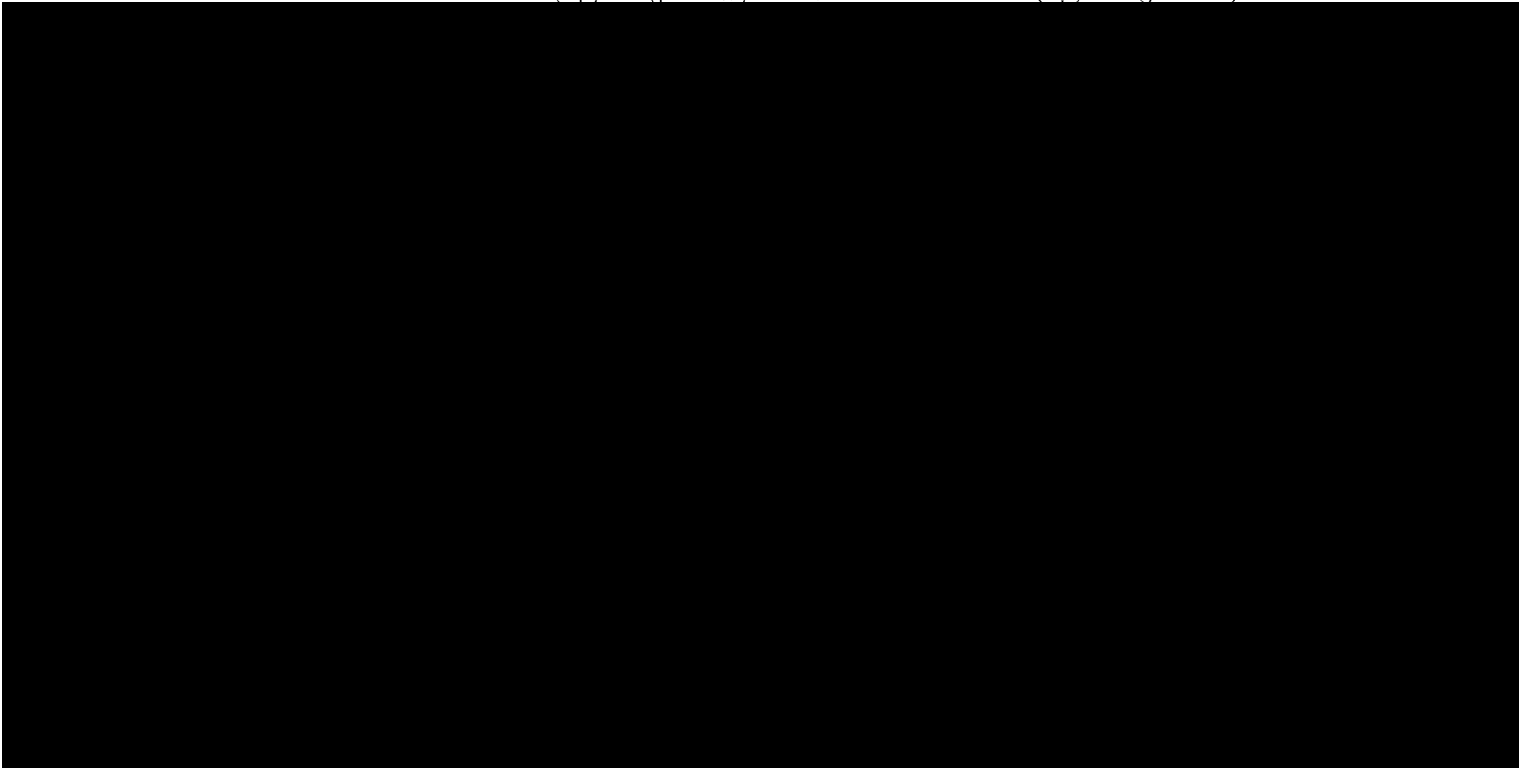
Table 16 Exon skipping efficiency by 4200TapeStation (PMO-R3 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-22-M-0661

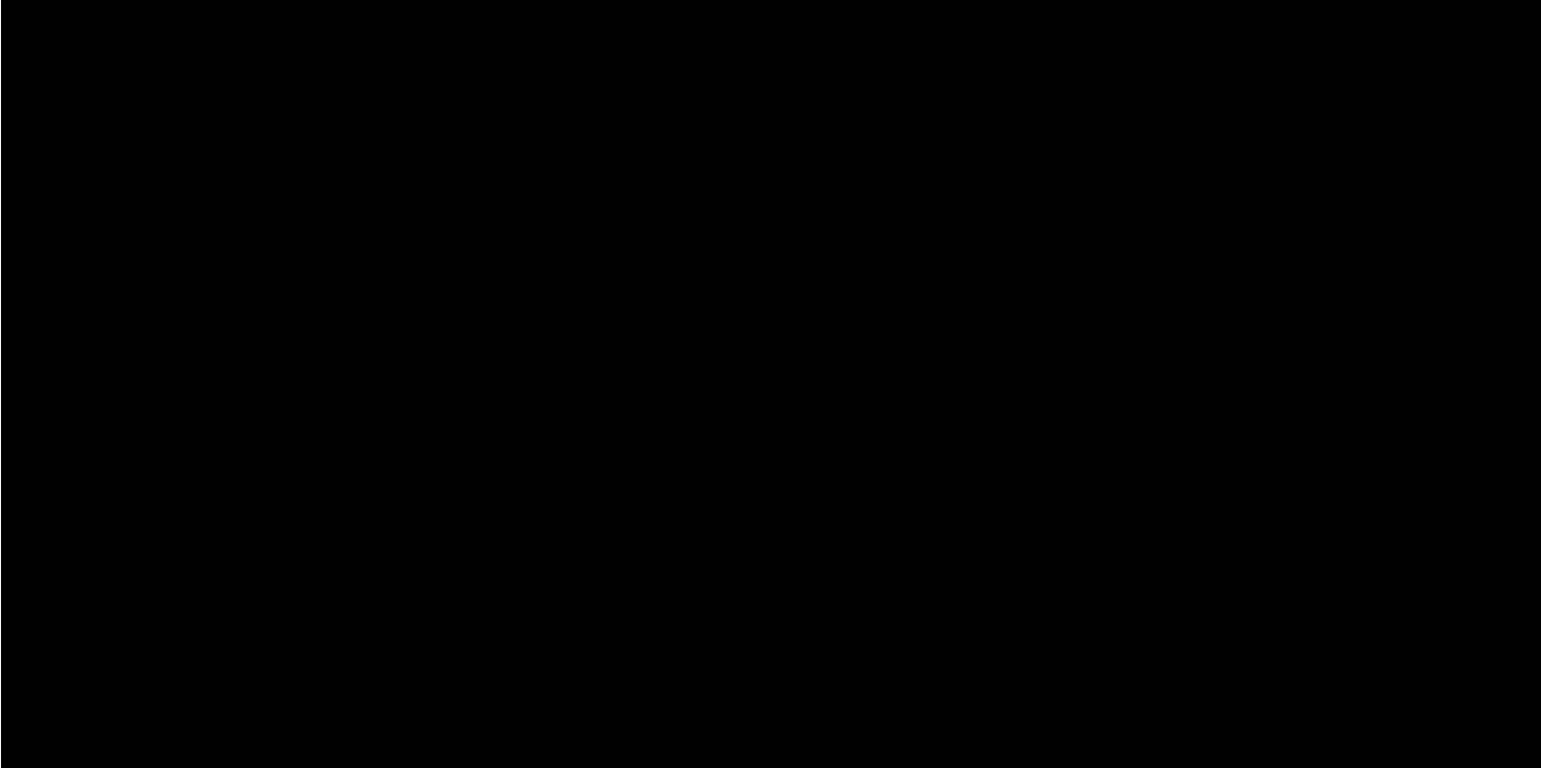
Table 17 Exon skipping efficiency by 4200TapeStation (PMO-R4 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-22-M-0661

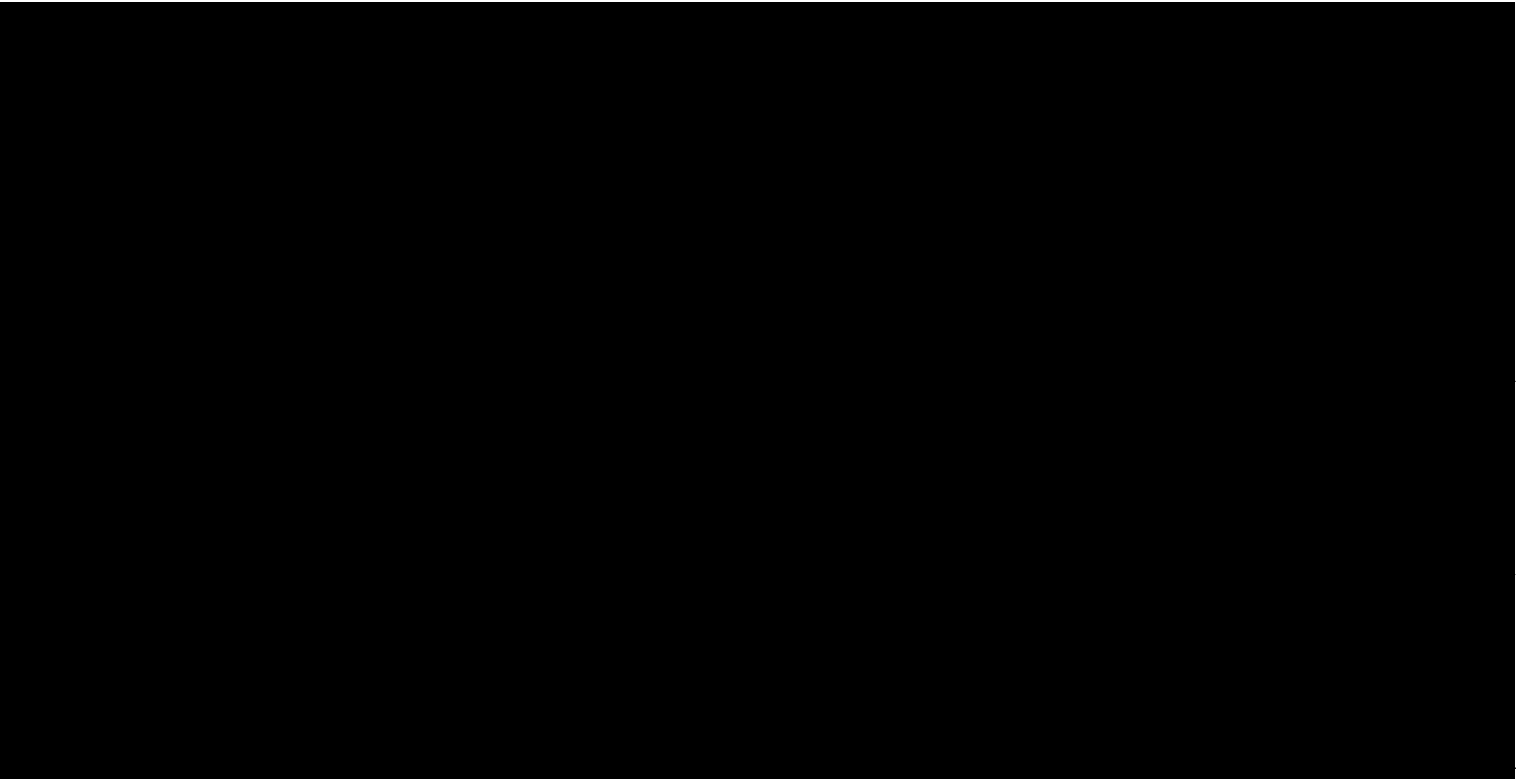
Table 18 Exon skipping efficiency by 4200TapeStation (PMO-R5 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (nmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (nmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

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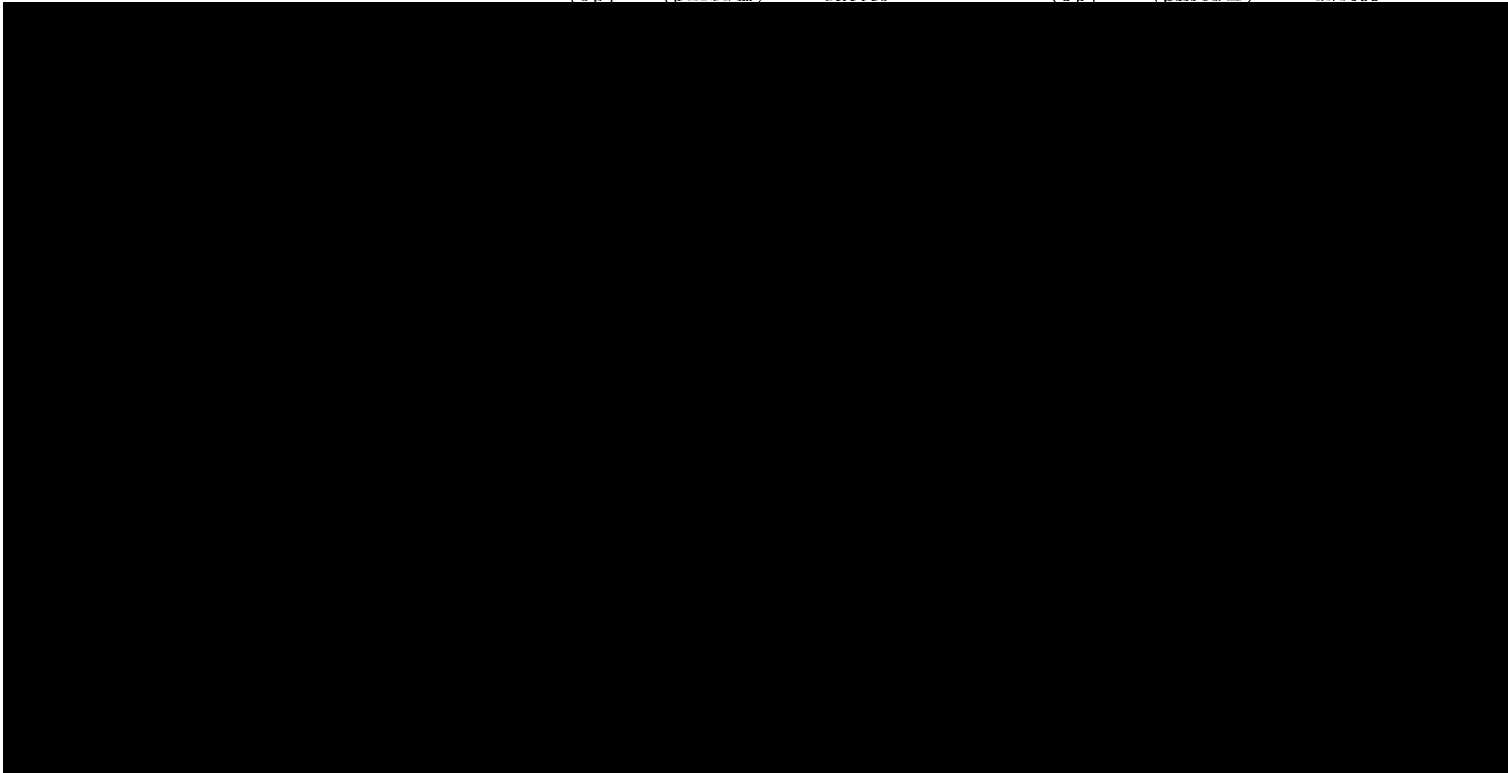
Table 19 Exon skipping efficiency by 4200TapeStation (PMO-R6 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

936-22-M-0661

Table 20 Exon skipping efficiency by 4200TapeStation (PMO-R7 exposed cells)

Exposure Sample	Exposure conc.	replicate	Size (bp)	Full length fragment Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Skipped fragment Conc. (pmol/L)	Dilution factor	Fig.	Skipping Efficiency
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable



8. STORAGE OF STUDY PLAN AND FINAL REPORT

The original study plan and final report are one copy and stored in the archives room (4207) of the testing facility. The duplicates of study plan and final report as PDF files are sent to the sponsor.

9. STORAGE AND RETENTION OF DOCUMENT AND RECORD

The original study plan, the original study plan amendment, the original final report, and other records concerning the study are stored in the archives room (4207) of the testing facility for 5 years after the study completion date. The test substances are stored in the archives room (4207) of the testing facility for 1 year after the study completion date. The management (disposal or return) of these items after the storage period will be notified the sponsor in writing before the completion of the storage period. After the storage period, a notice will be given in writing to the sponsor and the stored materials will be returned or disposed.

# Exhibit D

IN THE UNITED STATES DISTRICT COURT  
DISTRICT OF DELAWARE

<hr/>	)	
NIPPON SHINYAKU CO., LTD.,	)	
Plaintiff,	)	
	)	
v.	)	
	)	C.A. No. 21-1015 (MN)
SAREPTA THERAPEUTICS, INC.,	)	
Defendant.	)	
<hr/>	)	
SAREPTA THERAPEUTICS, INC. and	)	
THE UNIVERSITY OF WESTERN	)	
AUSTRALIA, Defendant and Counter-	)	
Plaintiff	)	
	)	
v.	)	
	)	
NIPPON SHINYAKU CO., LTD. and	)	
NS PHARMA, INC., Plaintiff and	)	
Counter-Defendants.	)	
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EXPERT REPORT OF DR. MICHELLE L. HASTINGS  
REGARDING INVALIDITY OF THE UWA PATENTS

September 8, 2023



Michelle L. Hastings, Ph.D.

86. In sum, my review of the UWA laboratory notebooks finds that the information on exon 53 skipping reported in Table 39 were selected from inconsistent results or were unfounded, given that there was no evidence of experimental testing in the provided notebooks.

87. My examination of the UWA laboratory notebooks strengthens my view that the inventors were not in possession of a genus of antisense oligonucleotide sequences at the time the UWA Patents were filed that were capable of inducing exon 53 skipping as claimed in the UWA Patents. The data omitted from the patent, were they included—especially Ms. Adam’s June 21, 2005 Experiment 42 showing that a PMO targeting H53A(+23+47) did not induce skipping at 20  $\mu$ M and 5  $\mu$ M—would only further reinforce my opinion that a POSA would not have understood that the named inventors possessed the invention they claimed in the UWA Patents.<sup>16</sup>

**E. Post-Filing Date Data Provides Further Evidence that UWA Was Not in Possession of a Sufficient Number of Species at the Alleged Priority Date**

**1. European Opposition Experimental Data**

88. I understand that NS challenged a European patent (EP 2206781 B1) at the European Patent Office in an Opposition proceeding, which stems from the same patent application that led to the UWA Patents. Claim 1 of that European patent was directed to similar subject matter as the ’851 Patent, i.e., an “antisense oligonucleotide that binds to human dystrophin pre-mRNA wherein said oligonucleotide is 20 to 31 nucleotides in length and is an oligonucleotide

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<sup>16</sup> Dr. Wilton tried to explain away the results of Ms. Adams’s Experiment 42, stating that the cells “were transfected; and they were harvested the next day. ... The morpholinos need more time. ... If those cells had been left longer, we now know we would have seen skipping.” Wilton Dep. Tr. at 175:12-24. But as Dr. Wilton confirmed, they did not know that transfection was an issue until well after the June 28, 2005 priority date of the UWA Patents. Thus, the results of Experiment 42 would have been understood by a POSA (*and the named inventors*), at the time the experiment was conducted, to mean that a PMO targeting H53A(+23+47) does not induce skipping at 20  $\mu$ M and 5  $\mu$ M. It was therefore disingenuous of the inventors to not include this data in the specification and later pursue patent claims directed to a PMO targeting this very region.

that is specifically hybridizable to an exon 53 target region of the Dystrophin gene designated as annealing site H53A (+23+47), annealing site H53A (+39+69), or both.” In response to certain of NS’s arguments in the proceeding, UWA submitted a declaration by Dr. Fred Schnell, dated April 25, 2018 (SRPT-VYDS-0228268), containing post-filing date data of several antisense oligonucleotides that fall within the scope of the UWA Patent claims. Tables 1 and 2 of the Schnell Declaration are reproduced below and provide a summary of antisense oligonucleotides 18 to 23 nucleotides in length that exhibit exon 53 skipping activity:

**Table 1: Experiment 1 Exon 53 Skipping Experimental Results**

Compound Name	Length	Dose					
		10µM		5µM		2.5µM	
		Mean	SD	Mean	SD	Mean	SD
Comparator H53A(+45+62)	18	2.9	2.7	0.5	0.9	0.6	1.0
AON2 (+32+51)	20	9.6	2.3	6.7	0.6	5.5	2.0
AON2 (+37+56)	20	17.2	4.7	10.8	4.9	5.8	1.1
AON3 (+40+59)	20	7.3	4.1	2.8	2.7	0.9	1.6
AON4(+36+56)	21	20.8	11.5	9.8	3.1	8.2	4.1
AON5 (+23+44)	22	11.3	7.0	5.9	1.1	4.3	2.1
AON6 (+29+50)	22	6.7	0.3	4.4	0.3	2.1	0.8
AON7 (+38+59)	22	2.5	2.3	3.3	0.2	0.8	1.4
AON8 (+41+62)	22	2.4	2.1	2.1	1.8	0.0	0.0
AON9 (+44+65)	22	0.7	1.0	0.0	0.0	0.0	0.0
AON10 (+32+54)	23	10.3	1.5	5.7	1.0	1.8	2.6
AON11 (+47+69)	23	0.0	0.0	0.0	0.0	0.0	0.0

**Table 2: Experiment 2 Exon 53 Skipping Experimental Results**

Compound Name	Length	Dose					
		10 $\mu$ M		5 $\mu$ M		2.5 $\mu$ M	
		Mean	SD	Mean	SD	Mean	SD
Comparator	18	4.5	2.2	2.2	1.2	0.8	1.1
H53A(+45+62)							
AON4(+36+56)	21	21.6	4.3	17.5	6.7	16.3	0.2
AON12 (+23+42)	20	13.7	2.3	7.2	2.2	2.1	2.9
AON13 (+26+45)	20	12.7	3.5	4.0	2.2	0.0	0.0
AON14 (+29+48)	20	10.8	0.0	5.2	1.1	2.9	1.2
AON15 (+38+57)	20	3.6	3.2	4.5	4.1	1.0	1.7
AON16 (+41+60)	20	7.4	3.5	2.5	2.4	5.1	3.8
AON17 (+44+63)	20	2.9	2.7	3.7	0.2	1.0	1.8
AON18 (+47+66)	20	0.0	0.0	0.5	0.9	0.0	0.0
AON19 (+23+43)	21	11.2	4.5	8.1	4.0	7.9	1.4
AON20 (+26+46)	21	16.0	24.4	8.2	7.2	1.6	2.2
AON21 (+29+49)	21	7.9	0.4	3.2	0.8	1.1	1.6
AON22 (+32+52)	21	18.4	4.5	5.4	4.8	6.3	2.0
AON23 (+38+58)	21	9.6	5.8	0.0	0.0	3.8	0.3
AON24 (+41+61)	21	5.5	1.6	1.7	3.0	1.2	2.0
AON25 (+44+64)	21	0.6	1.0	0.0	0.0	0.0	0.0
AON26 (+46+66)	21	0.0	0.0	0.0	0.0	1.4	2.4
AON27 (+31+53)	23	10.6	5.6	6.4	6.0	2.8	4.0
AON28 (+36+58)	23	15.2	3.1	4.8	4.9	5.2	2.0
AON29 (+39+61)	23	3.3	3.1	2.6	2.3	2.5	2.8
AON30 (+40+62)	23	5.9	1.3	3.4	3.9	2.4	4.1
AON31 (+45+67)	23	0.0	0.0	0.0	0.0	0.0	0.0
AON32 (+46+68)	23	0.6	1.0	3.0	2.6	0.0	0.0

89. Several of the antisense oligonucleotides that Dr. Schnell tested fall within the scope of the UWA Patent claims, including antisense oligonucleotides 2, 4, 6, 10, 14, 18, 21, 22, 27 and 28. It is my understanding that such post-filing date data, however, cannot be used to affirmatively satisfy the written description requirement as written description requires a specification to reasonably convey to those skilled in the art that the inventor had possession of the claimed subject matter as of the alleged priority date. The data in the Schnell declaration is not disclosed in the UWA Patents, and therefore, cannot convey possession of the claimed subject matter at of the alleged priority date. The fact that UWA was compelled to test additional species

after-the-fact in the European Opposition proceeding further shows that the inventors failed to disclose a representative number of species in the specification of the UWA Patents.

## **2. Chemicals Evaluation and Research Institute (CERI) Experimental Reports**

90. While evaluating the sufficiency of the UWA Patents' written description and enablement, I considered whether additional testing would be informative to the inquiries, *e.g.*, the disclosure of a representative number of species, the existence of a structure-function relationship and the predictability of exon-skipping antisense oligonucleotides directed to exon 53. I have been informed by counsel and understand that, although the written description inquiries apply the lens of a POSA at the time of the inventions, post-priority date testing may be relied upon as evidence of such factors.

91. Accordingly, I designed protocols for experiments ultimately performed by CERI at my direction, which are described in the three CERI Reports. *See generally* NS00102924 (Study Number: 936-21-M-0643); NS00102988 (Study Number: 936-21-M-0644); NS00103061 (Study Number: 936-22-M-0661).

92. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Like many contract research organizations, CERI offers “state-of-the-art facilities” and “highly qualified professionals” who “conduct a range of chemical analysis techniques and bio-testing methods”:

As an independent, unbiased organization that is committed to keeping chemicals and the environment in balance, CERI plays a supporting role in the research and development projects of clients utilizing its services. Working in state-of-the-art facilities, our highly qualified professionals conduct a range of chemical analysis

techniques and bio-testing methods for these clients. CERI's staff has also performed tests, analysis, research, and studies designed to solve technical problems in the field of chemicals.

See [https://www.cerij.or.jp/ceri\\_en/gaiyou/gaiyou\\_menu.html](https://www.cerij.or.jp/ceri_en/gaiyou/gaiyou_menu.html). The experiments I designed were carried out by CERI's Chemicals Assessment and Research Center, which has experience developing "novel methodology" relating to "cell based assay and other in vitro methodologies," as well as performing "[j]oint research with partners in industry, academia, and the government."

See [https://www.cerij.or.jp/ceri\\_en/gyoumu/hyoukaken\\_menu.html](https://www.cerij.or.jp/ceri_en/gyoumu/hyoukaken_menu.html). In my opinion, [REDACTED]

[REDACTED]

93. CERI had suitable equipment and could source suitable reagents for the experimental protocols I designed, each of which is described in the "Materials and Methods" section of the corresponding CERI Report. [REDACTED]

[REDACTED] Cells were therefore sourced from the manufacturer Lonza and provided to CERI.

**a. Study Number: 936-21-M-0643**

94. [REDACTED]

[REDACTED] I decided to conduct this testing because, in my opinion, a POSA would find the specification lacking for actual data on SEQ ID No. 195 and other exon 53-directed AOs. Apart from SEQ ID NO: 193, the figures do not show any testing of individual exon 53-directed antisense oligonucleotides. '851 Patent at Figure 22. In my opinion, a POSA reviewing the specification would understand Table 39 as reporting on the activity of the individual antisense oligonucleotides listed. However, the only objective data provided for SEQ ID NO: 195 is as part of a cocktail with



SEQ ID NOs: 194 and 196.<sup>17</sup> *Id.* at Figure 22; 64:41-45. Although the specification discusses exon 53 cocktails and weasels separately in Table 1B and Table 1C, Table 39 reports the exact same “[a]bility to induce skipping” for each antisense oligonucleotide used in the cocktail test: SEQ ID NOs: 194, 195 and 196:

TABLE 39

SEQ ID	Antisense oligonucleotide name	Sequence	Ability to induce skipping
		*	*
194	H53D (+14-07)	UAC UAA CCU UGG UUU CUG UGA	Very faint skipping to 50 nM
195	H53A (+23+47)	CUG AAG GUG UUC UUG UAC UUC AUC C	Very faint skipping to 50 nM
196	H53A (+150+176)	UGU AUA GGG ACC CUC CUU CCA UGA CUC	Very faint skipping to 50 nM

95. This led me to question whether the activity reported in Table 39 for SEQ ID NOs: 194, 195 and 196, was based on testing the cocktail discussed in the specification. I therefore designed an experimental protocol to test those three antisense oligonucleotides both individually and as a cocktail.<sup>18</sup> Because my goal was to evaluate the UWA Patents’ disclosures with this

<sup>17</sup> The specification’s disclosures concerning exon 53 antisense oligonucleotides are rife with errors. The specification, for example, states that Figure 22 shows testing of “H53A(+39+69) [SEQ ID NO:193],” but Figure 22 mislabels this ASO as “H53D(+39+69).” *See* ’851 Patent at 64:38-41. The specification’s text also describes “H53A(+39+69) [SEQ ID NO:193]” as being tested at a 5 nM concentration, while Table 39 only describes the “[a]bility to induce skipping” down “to 50 nM.” *See id.* Additionally, the specification describes the cocktail test as being shown by Figure 20, but Figure 20 is labeled as relating to H42A(-4+23). *See id.* at 64:41-45, Fig. 20. Figure 22 appears to show the “Cocktail” test for exon 53, but the antisense oligonucleotides are all labeled as “H53D,” when SEQ ID NOs: 195 and 196 should be “H53A.” The extent of these errors would further detract from a POSA’s confidence in the robustness of the results reported. In my experience in this field, such extensive, glaring errors in reporting one’s results would be unacceptable for peer-reviewed literature and would have to be corrected before publication.

<sup>18</sup> In my experience, seeking to replicate another’s previously-reported results is commonplace in scientific fields as a confirmatory check on data. *See also* Adams Dep. Tr. at 32:2-14 (discussing

experiment, I selected the same antisense oligonucleotide backbone (2'-O-methyl) as used in the UWA Patent examples. *See, e.g.*, '851 Patent at 32:33-47 ("2OMe antisense oligonucleotides were designed to be complementary to the target sequences under investigation and were synthesized on an Expedite 8909 Nucleic Acid Synthesiser. . . . Specified amounts of the antisense molecules were then tested for their ability to induce exon skipping in an in vitro assay, as described below.")). Likewise, I selected normal human muscle cells because the specification's examples did so. *Id.* at 32:48-50. The selected concentrations (300 nM and 600 nM) of 2'-O-methyl antisense oligonucleotides were also used in the specification's examples for 2'-O-methyl antisense oligonucleotides for exon 53. *See id.* at 64:32-50.

96. The specification generically states that the named inventors performed cell culturing, transfection, RNA extraction, and RT-PCR to obtain their data, but does not describe any of the experimental procedures or techniques employed that would enable a POSA to replicate them exactly. *See, e.g.*, '851 Patent at 32:48-60. I therefore modeled my experimental protocol largely after the named inventors' methods as described in Dr. Wilton's and Dr. Fletcher's publications from around the year 2005, namely Errington, Stephen J., et al. "Target selection for antisense oligonucleotide induced exon skipping in the dystrophin gene." *The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications* 5.6 (2003): 518-527, and Harding. Where necessary, I used my professional judgment to select appropriate modifications, such as in identifying suitable reagents to replace those identified in Errington or Harding that were no longer commercially available. As one example, I

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desire to compare AOs she had made to see how they compared to Van Deutekom's published AO sequence), 89:20-90:2 (testifying "of course [Popplewell] can use [Wilton's oligo] as a reference"), 99:11-19 (describing how she attempted to independently verify Popplewell's results).

decided to use the RT-PCR primers reported in Sarepta's U.S. Patent Application Publication No. 2010/0130591 A1 to Sazani and Kole for detecting exon 53 skipping in human dystrophy (*see* tbl. 1) instead of those reported in Harding because they generate shorter PCR products. The percent skipping for each PCR product was calculated by the following formula:

NS00102937.<sup>19</sup>

97. A summary of the tested 2'-O-methyl antisense oligonucleotides is provided in the table below. I selected the UWA Patents' self-described "strongest" antisense oligonucleotide at inducing exon 53 skipping (SEQ ID NO: 195) as the positive control. *See* '851 Patent at 64:49-50. The specification did not identify any antisense oligonucleotide negative controls, so I used "blanks" (*i.e.*, 0 nM concentrations) as a negative control.

<sup>19</sup> The same formula was used in each of the three CERI reports.

<sup>20</sup> ASO = antisense oligonucleotide

<sup>21</sup> If listed relative to the exon 53 donor site, the coordinates would be H53D(+14-07).

<sup>22</sup> The results for ASO-4 are not consistent with the results reported in Table 39 of the UWA Patents for SEQ ID NO: 195. It is possible that this is due to the "extra U" that may have been incorporated into the Wilton laboratory's JSR 543 oligonucleotide. *See* Section IX.D., above.

98. As shown in the tables below, [REDACTED]

[REDACTED] These individual results (which vary across the three cocktail antisense oligonucleotides) are inconsistent with how Table 39 of the UWA Patents describes their “Ability to induce skipping” (identically across all three as “Very faint skipping to 50 nM”).

99. My results are not inconsistent with a conclusion that Table 39 reports the activity of a SEQ ID NOs: 194, 195, and 196 cocktail, *not* each antisense oligonucleotide tested individually. If a POSA conducted this confirmatory experiment and obtained the results that I did, they might conclude that the exon skipping activity of SEQ ID NO: 195 reported in Table 39 was based on its performance in a cocktail.<sup>23</sup> This would further support my opinion that a POSA would not understand the named inventors to have possessed the claimed genus of PMOs at the time of the invention. Because the activity of individual antisense oligonucleotides within a cocktail cannot be determined or predicted from the activity of the cocktail, a POSA would *not* understand that SEQ ID NO: 195 induces exon-skipping individually based on the activity of the cocktail. *See, e.g.*, Adams 2007 (“Some AOs were inactive when applied individually, yet pronounced exon excision was induced in transfected cells when the AOs were used in select combinations”). As such, the specification would certainly not provide a POSA any basis to conclude that the inventors possessed an entire genus of exon 53-skipping PMOs having as few as a mere 12 consecutive bases of SEQ ID NO: 195 based on the “faint skipping at 50 nM” induced by a “cocktail” containing SEQ ID NO: 195.

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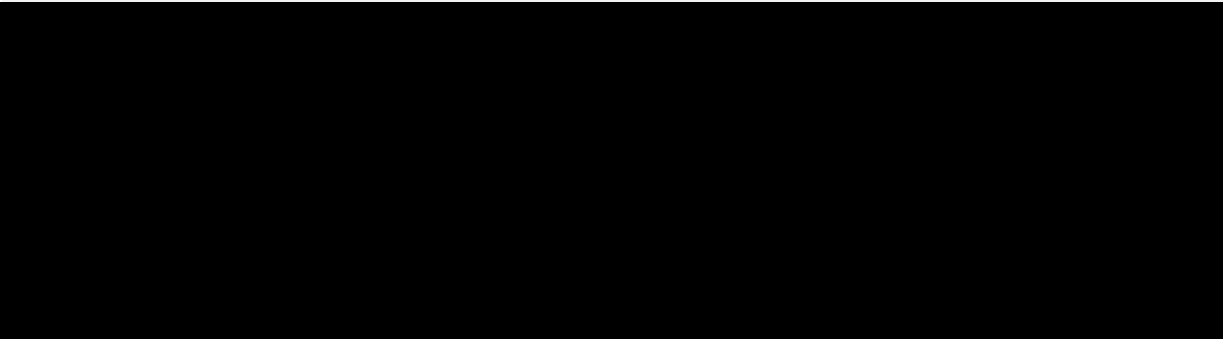
<sup>23</sup> Of course, a POSA would not have access to the UWA laboratory notebooks, which establish that the “cocktail” in Figure 22 was actually a weasel.



100. A more detailed summary of the results obtained from this study is presented in the tables below.

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

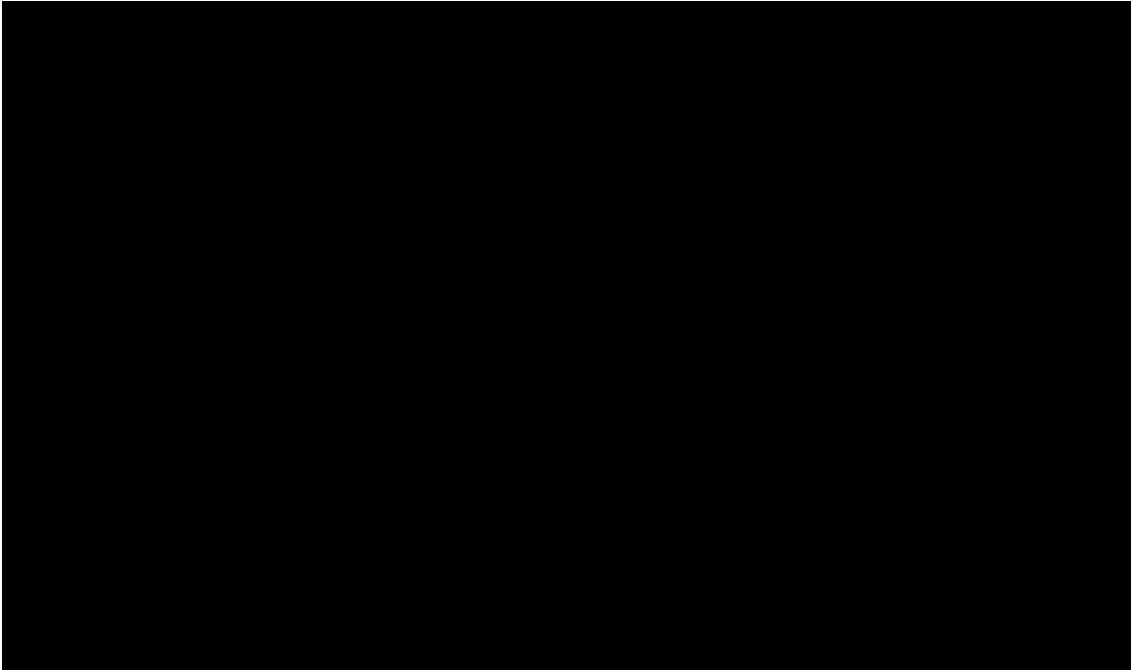


Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											



											
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	

											N/A
											N/A
											N/A
											
											N/A
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size	Conc.	Dilution	Fig.	Size	Conc.	Dilution	Fig.	



	(bp)	(pmol/L)	factor	(bp)	(pmol/L)	factor
						N/A
						N/A
						N/A
						N/A
						N/A
						N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A
											N/A
											N/A






n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, *N/A*: Not Applicable

**b. Study Numbers: 936-21-M-0644 and 936-22-M-0661**

101. Study Nos. 936-21-M-0644 (NS00102988) and 936-22-M-0661 (NS00103061)

 I designed these experiments to provide more data regarding whether and how exon skipping activity varies across the variety of different PMOs meeting the structural limitations of the claimed genus. The PMOs I selected   




[REDACTED]

102. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] As discussed below, these results provided helpful information demonstrating the unpredictability of exon-skipping across PMOs having the claimed structural features and the lack of structure-function relationship between exon 53-skipping and the claimed structural features.

103. Because my goal was to evaluate the claimed genus, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

104. Again, I modeled my experimental protocol largely after the named inventors' methods as described in Dr. Wilton's and Dr. Fletcher's publications from near 2005, as noted above (Study No. 936-21-M-0643). Where necessary, I used my professional judgment to select

[REDACTED]

appropriate modifications, such as in identifying suitable reagents to replace those identified in Errington or Harding that were no longer commercially available. As an example, I decided to use a more modern transfection technique (with Endo-Porter) than that disclosed in Harding that I felt would better ensure that the PMOs would be delivered to cells (*i.e.*, to avoid false negatives).<sup>24</sup> Again, I decided to use the RT-PCR primers reported in Sarepta's U.S. Patent Application Publication No. 2010/0130591 A1 to Sazani and Kole for detecting exon 53 skipping in human dystrophy (*see* Table 1) instead of those reported in Harding, because they would provide a shorter PCR product.

(1) Study No. 936-21-M-0644

105. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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<sup>24</sup> I also consulted Summerton, James, and Dwight Weller. "Morpholino antisense oligomers: design, preparation, and properties." *Antisense and Nucleic Acid Drug Development* 7.3 (1997): 187-195, and considered its scrape-loading method, but ultimately decided to proceed with the more modern Endo Porter technique.

[REDACTED]

b [REDACTED]  
[REDACTED]  
[REDACTED]  
[REDACTED]  
[REDACTED]  
[REDACTED].

Study No. 936-21-M-0644				
Tested PMO	Length	Position on Exon 53	Skipping	Notes
[REDACTED]				

107. [REDACTED]  
[REDACTED]

<sup>25</sup> Does not fall within the scope of the UWA Patent claims.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] These

data show that there is no structure-function relationship between exon 53-skipping and the structural features claimed in the UWA Patents.

108. A more detailed summary of the results obtained from this study is presented in the tables below.

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		

n.d.: not detected, N/A: Not Applicable



Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (nmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (nmol/L)	Dilution factor	Fig.	

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

*N/A*

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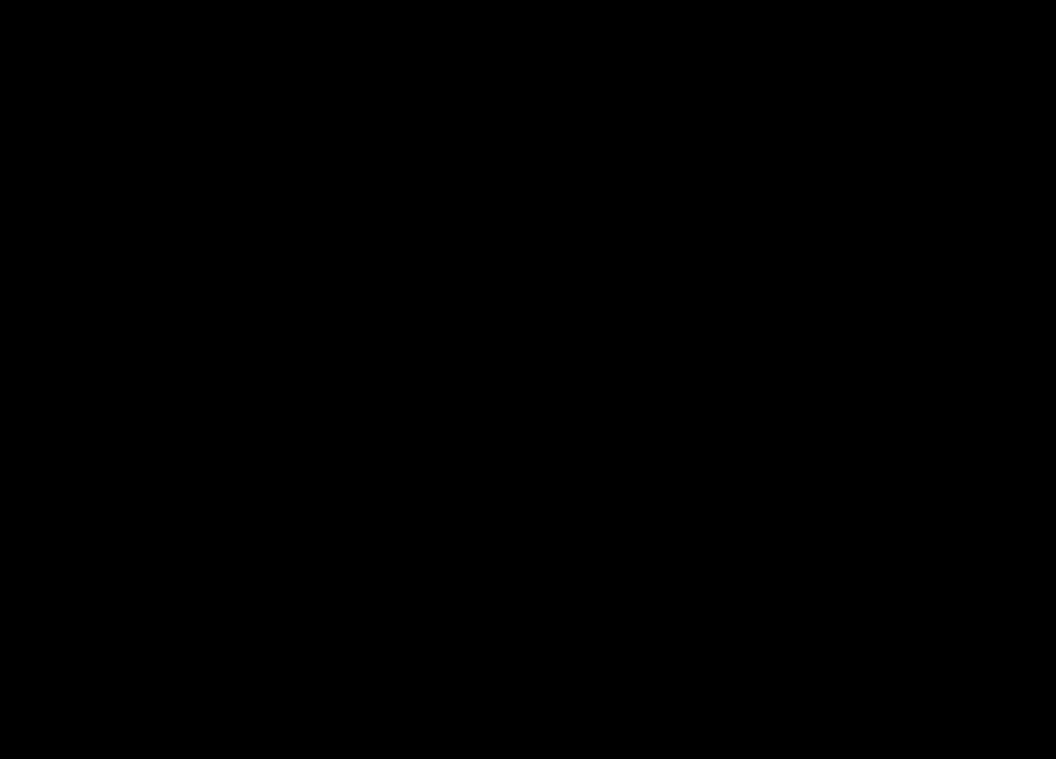



1. *Journal of the American Medical Association*, 2000; 283: 2639-2645.

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										N/A
										N/A
										N/A
										N/A
										N/A
										N/A
										N/A
										N/A
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										N/A

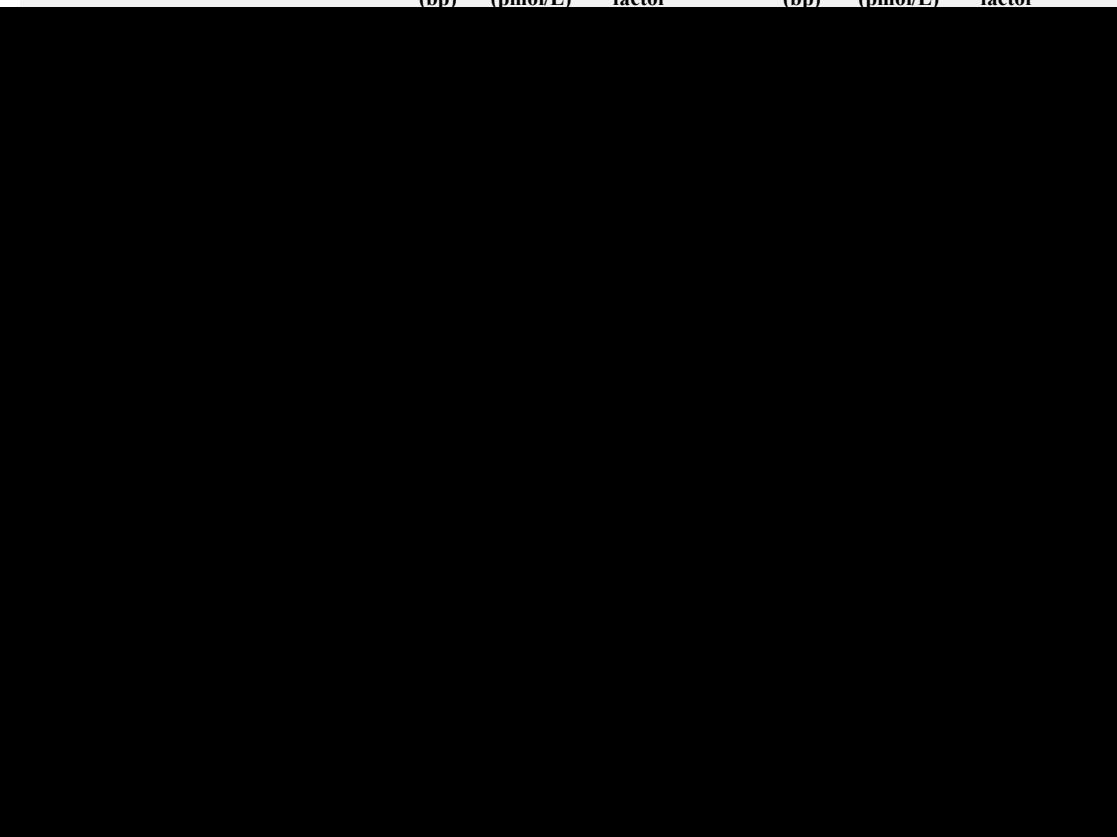
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Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A
											N/A
											N/A
											
											
											N/A
											



3	387	2,300	40	(24)	160	228	1	(23)	0.2
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n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A





n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Size (bp)	Skipped fragment		Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor			Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Size (bp)	Skipped fragment		Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor			Conc. (pmol/L)	Dilution factor		
											N/A

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											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
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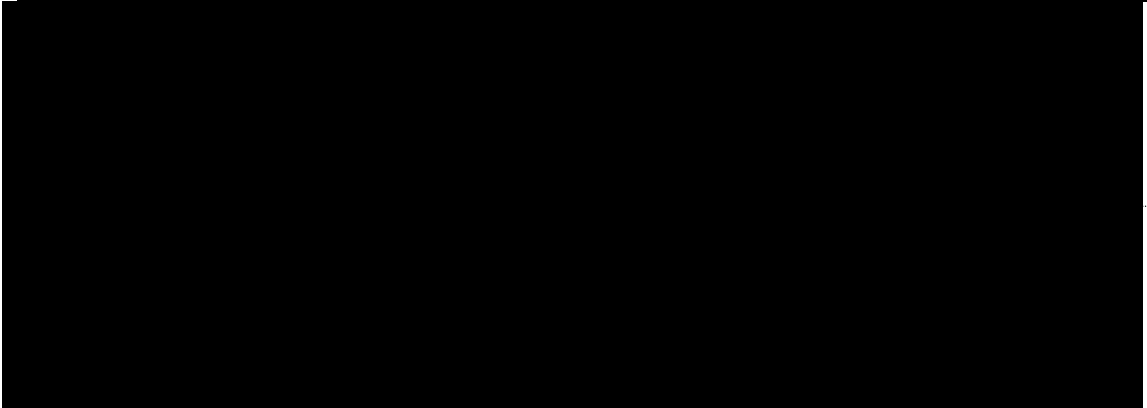




		3	384	3,310	40	(44)	169	334	40	(44)	9.2
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n.d.: not detected, *N/A*: Not Applicable


Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A

n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A
											
											N/A
											



n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A
											N/A
											N/A
											

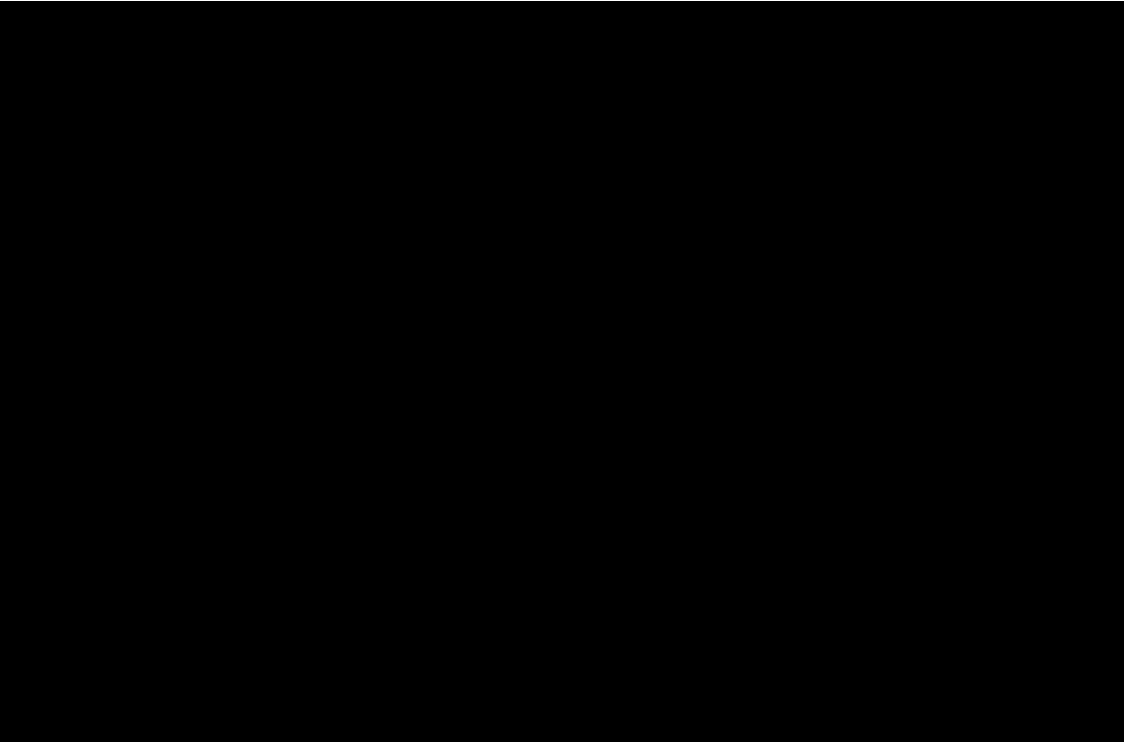
n.d.: not detected, *N/A*: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A



											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

[REDACTED]

3	388	2,020	40	(72)	154	528	1	(70)	0.6
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n.d.: not detected, N/A: Not Applicable

(2) Study No. 936-22-M-0661

109. The purpose of this study was to evaluate whether “weasel” antisense oligonucleotides comprising “at least 12 consecutive bases of” SEQ ID NO: 195 would induce exon 53 skipping. The UWA Patents define a “weasel” as “a cunningly designed antisense oligonucleotide” that is made “by joining together two or more antisense oligonucleotides molecules.” *See* ’851 Patent at 4:56-61. I understand that the Court has expressly construed the UWA Patent claims as encompassing “weasels.” D.I. 248 at 10-11.

110. Again, a PMO [REDACTED] was selected as a positive control. A PMO t [REDACTED] was selected as a negative control. [REDACTED]


[REDACTED]

[REDACTED]


[REDACTED].

Study No. 936-22-M-0661				
Tested PMO	Length	Position on Exon 53	Skipping	Notes
[REDACTED]				

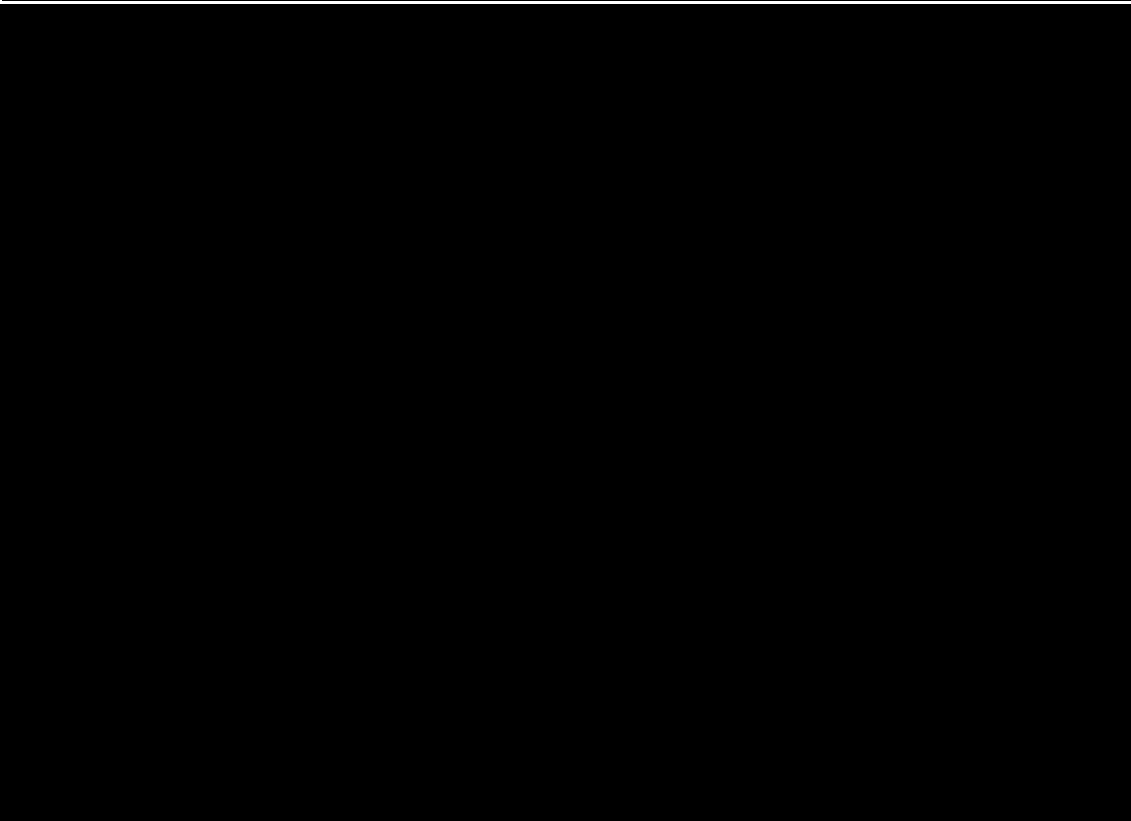



111. As summarized above, despite each containing at least 12 consecutive bases of SEQ ID NO: 195, not all of the PMOs induced exon 53 skipping. For example, 



 These data are further evidence of the lack of structure-function relationship between exon 53-skipping and the structural features claimed in the UWA Patents.

112. A more detailed summary of the results obtained from this study is presented in the tables below.

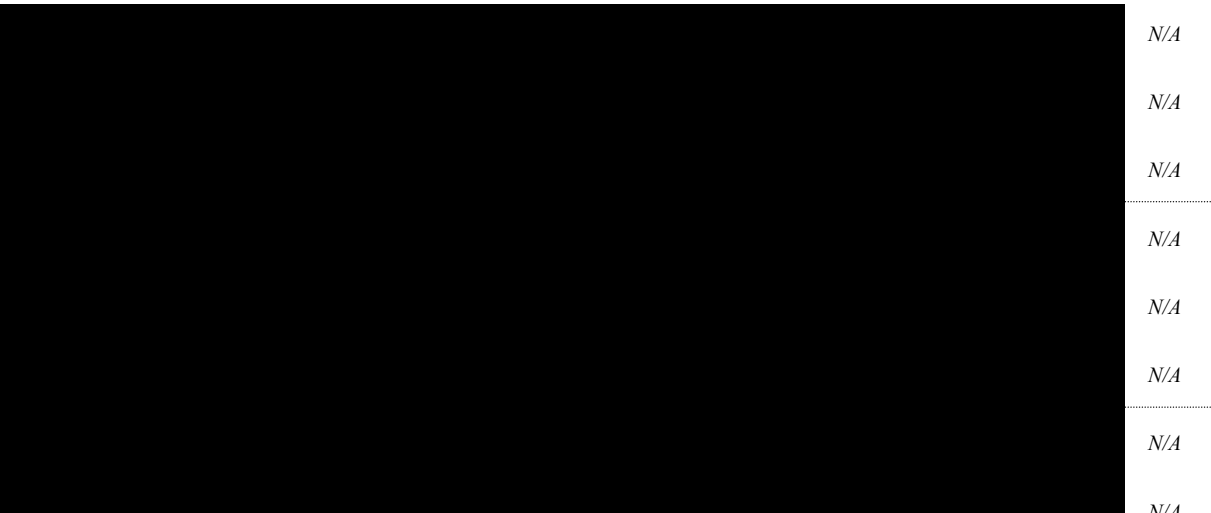
Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable



Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
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											N/A
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											N/A
											N/A
											N/A
											N/A
											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A



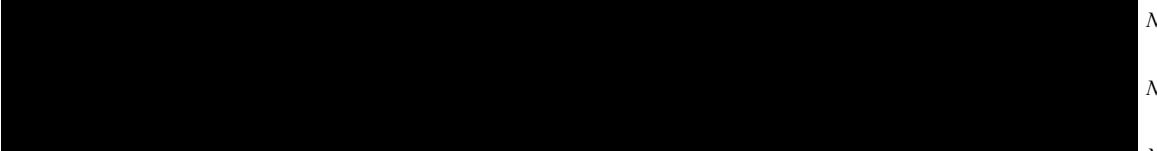


										N/A
										N/A
										N/A
										N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment				Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
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											N/A
3.2											
3.3											
5.2											

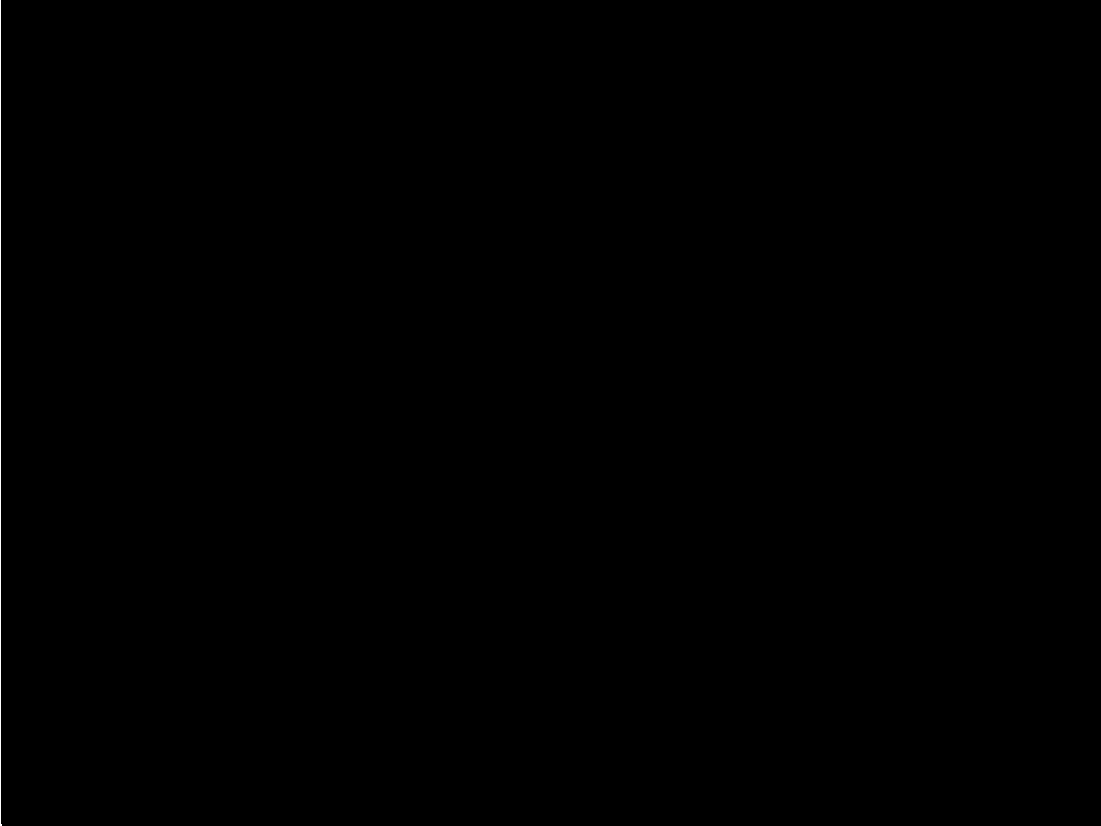

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment			Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	
										N/A
										N/A
										N/A



										N/A
										N/A
										N/A
										N/A
										N/A
										N/A
										N/A
										N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											N/A
											

n.d.: not detected, N/A: Not Applicable



Exposure Sample	Exposure conc.	replicate	Full length fragment			Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor		Size (bp)	Conc. (pmol/L)	Dilution factor		
											N/A
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											N/A
											N/A
											N/A
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											N/A
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											N/A
											N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Skipped fragment			Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor	Fig.	Size (bp)	Conc. (pmol/L)	Dilution factor	
										N/A
										N/A
										N/A
										N/A
										N/A
										N/A
										N/A
										N/A

[REDACTED]

[REDACTED]	N/A
	N/A
	N/A
	N/A

n.d.: not detected, N/A: Not Applicable

Exposure Sample	Exposure conc.	replicate	Full length fragment				Fig.	Skipped fragment			Fig.	Skipping Efficiency
			Size (bp)	Conc. (pmol/L)	Dilution factor			Size (bp)	Conc. (pmol/L)	Dilution factor		
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	N/A
												N/A
												N/A
												N/A
												N/A
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												N/A
												N/A
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												N/A
												N/A

n.d.: not detected, N/A: Not Applicable

113. Although the CERI reports were generated after the alleged June 2005 priority date of the UWA Patents, I understand that post-priority-date evidence can be considered where, as here, it is used to evaluate whether the disclosed species sufficiently represent the claimed genus. As shown above, [REDACTED]

[REDACTED] induce exon 53 skipping. The results of

the CERI reports further support my opinion that a POSA, reading the specification of the UWA Patents, would not conclude that it sufficiently discloses the claimed genus of antisense oligonucleotides.

**X. THE UWA PATENTS ARE NOT ENABLED**

114. Practicing the full scope of the UWA Patent claims would require undue experimentation, particularly in view of their breadth, the unpredictability of the art, the lack of guidance and working examples provided in the UWA Patents, and the vast amount of time-consuming experimentation that would be required to test each and every member of the broadly claimed genus for exon 53 skipping activity.

**A. The Breadth of the UWA Patent Claims**

115. As discussed above, the UWA Patent claims encompass up to  $10^{14}$  antisense oligonucleotide sequences. The claims also encompass antisense oligonucleotides with a wide range of exon 53 skipping activity, including antisense oligonucleotides having “very faint skipping” activity to antisense oligonucleotides with therapeutic effect. Thus, the number of potential antisense oligonucleotides that one would need to screen to determine whether that antisense oligonucleotide falls within the scope of the claim is enormous.

**B. The Nature of the Invention and the Unpredictability in the Art**

116. As discussed by Dr. Wood, the ability of antisense oligonucleotides to induce dystrophin pre-mRNA exon skipping is unpredictable. Wood Report ¶¶ 75-89; *see also* Wood Interference Declaration ¶¶ 68-81. Indeed, as stated by the named inventors in the specification of the UWA Patents:

Attempts by the inventors to develop a rational approach in antisense molecules design were not completely successful as there did not appear to be a consistent trend that could be applied to all exons. As such, the identification of the most effective and therefore

scope of the claimed invention would have required synthesizing and testing each of these antisense oligonucleotides. This would have required tremendous and undue experimentation.<sup>27</sup>

123. Indeed, members of Dr. Wilton's laboratory had difficulty reproducing data showing exon 53 skipping. *Compare* SRPT-VYDS-0156085 at 6212 (Experiment 21) *with* SRPT-VYDS-0158109 at 8154 (Experiment 43); *see also, e.g.,* SRPT-VYDS-0156085 at 6089 (describing results as "not consistent"); Wilton Dep. Tr. at 174:12-177:2 (admitting skipping results were inconsistent and not dose-dependent). Even in 2009, four years after the alleged June 2005 priority date, members of Dr. Wilton's laboratory could not reproduce data showing exon 53 skipping. Adams Dep. Tr. at 64:1-65:8 (describing multiple potential explanations for inconsistent results); Meloni Dep. Tr. at 107:20-109:26 (describing multiple potential explanations for inconsistent results); SRPT-VYDS-0159885 at 903 (unable to show H53A(+39+69) induced skipping at low concentrations); SRPT-VYDS-0159207 at 268 and 270 (inconsistent gels for AVI P007 AO-53 +33+64); SRPT-VYDS-0159343 at 9357 ("It is clear that no AO has resulted in anything like 50% skipping. I have therefore been unable to repeat the results previously generated in the laboratory.").<sup>28</sup>

124. Moreover, as of the June 28, 2005 priority date, the inventors had not been able to induce exon 53 skipping with PMOs. *E.g.,* SRPT-VYDS-0158109 at 8145. Dr. Wilton attributed

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<sup>27</sup> The CERI Reports, which [REDACTED] of antisense oligonucleotides encompassed by the genus of the UWA Patent claims, took upwards of 14 months to complete. *See* NS00102924 at 926, 990; NS00103061 at 063.

<sup>28</sup> Sarepta's Final Validity Contentions rely on the testimony of Mr. Satou, who is a named inventor of the NS patents, to argue that "companies like NS with more resources were capable of testing many antisense oligonucleotides at once in a short period of time." Sarepta Final Validity Contentions at 36. I understand that Mr. Satou was testifying as to the 2009-2010 timeframe (which I understand is the relevant timeframe for the NS patents), whereas the undue experimentation here is judged as of the June 28, 2005 priority date of the UWA Patents. I am not aware of high-throughput techniques for screening libraries of antisense oligonucleotides for exon skipping that were available in 2005.

# Exhibit E

IN THE UNITED STATES DISTRICT COURT  
DISTRICT OF DELAWARE

<hr/>	)	
NIPPON SHINYAKU CO., LTD.,	)	
Plaintiff,	)	
	)	
v.	)	
	)	C.A. No. 21-1015 (GBW)
SAREPTA THERAPEUTICS, INC.,	)	
Defendant.	)	
<hr/>	)	
SAREPTA THERAPEUTICS, INC. and	)	
THE UNIVERSITY OF WESTERN	)	
AUSTRALIA, Defendant and Counter-	)	
Plaintiff	)	
	)	
v.	)	
	)	
NIPPON SHINYAKU CO., LTD. and	)	
NS PHARMA, INC., Plaintiff and	)	
Counter-Defendants.	)	
<hr/>		

REPLY EXPERT REPORT OF DR. MICHELLE L. HASTINGS  
REGARDING THE INVALIDITY OF THE UWA PATENTS

October 27, 2023





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Michelle L. Hastings, Ph.D.



**E. Response to Dr. Dowdy's Arguments Regarding the CERI Experiments**

**1. Study Number 936-21-M-0644**

41. Dr. Dowdy states that “[t]here are numerous problems with the ‘experimental’ oligonucleotides” that I designed for this study. Dowdy Rebuttal ¶ 204. Dr. Dowdy states that “  
 ‘antisense’ oligonucleotides, as a POSA would have understood from reading the claims of the Wilton Patents” because they contain “bases that are not complementary to exon 53 of the human dystrophin pre-mRNA.” *Id.* I disagree. As explained above, under the Court’s construction, only the “base sequence” needs to be 100% complimentary to the human dystrophin pre-mRNA and the base sequence need not span the entirety of the AO. *See* above, § V.A. Therefore, PMO-7, PMO-8, PMO-10, and PMO-11 are “antisense” oligonucleotides as recited in the claims.

42. Dr. Dowdy also complains that I purportedly did not follow the teaching of the UWA Patents to design the tested AOs. Specifically, Dr. Dowdy states that my AOs lack “a sufficient degree of complementarity or precise pairing” to exon 53 of the human dystrophin pre-mRNA. Dowdy Rebuttal ¶ 205. This language is not in the claims.<sup>22</sup> I specifically designed the AOs based on the language of the claims. And the AOs that I designed all fall within the scope of the claims, as construed by the Court. *See* above, § V.A.

**2. Study Number 936-22-M-0661**

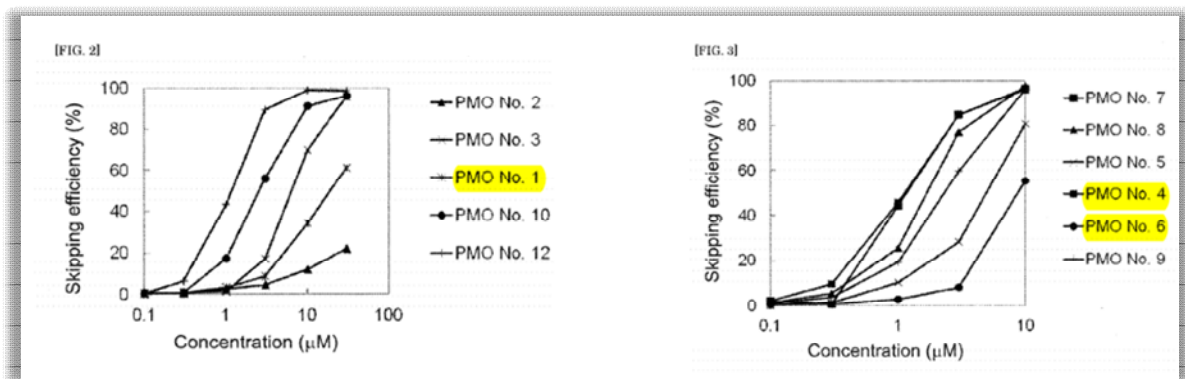
43. Dr. Dowdy raises similar complaints against the AOs that I designed for this study, including that they are not 100% complimentary to the human dystrophin pre-mRNA. He also

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<sup>22</sup> I understand that it is improper for Sarepta to advance a new claim construction at this stage in the proceeding.

complains that I designed the “weasels” contrary to the teachings of the UWA Patents. Dowdy Rebuttal ¶ 217. Specifically, Dr. Dowdy argues that “weasels tend to be very long, as they have independent segments that bind different regions of the target pre-mRNA, and can have additional, flexible nucleotides to serve as a linker.” *Id.* According to Dr. Dowdy, my “‘weasels’ are too short” so “they lack the length needed for efficient binding and the flexibility to bind disparate target regions of exon 53.” *Id.* Dr. Dowdy states that he has “not seen similar compounds reported in the scientific literature for exon skipping.” *Id.* However, there are plenty of examples of similar compounds in the literature.

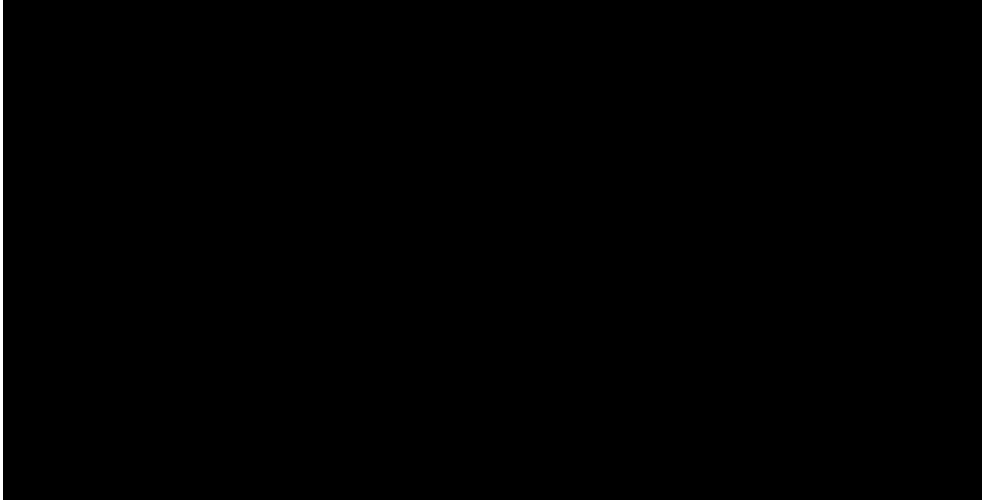
44. For example, U.S. Patent No. 9,840,706 (to NS) discloses “antisense oligomer[s] obtained by connecting oligomers targeting two different sites in exon 44 in the human dystrophin gene can induce skipping of this exon.” U.S. Patent No. 9,840,706 at 3:17-23. Thus, the ’706 patent discloses “weasel”-type AOs. Table 1 of the ’706 patent discloses various weasel AOs in a PMO backbone. *Id.* at 33:30 – 35:7. Most if not all of the weasels in Table 1 are 30 bases or less in length. *See* Sequence Listing. Moreover, some weasels target regions that sit almost 100 bases apart. Table 1 at *e.g.*, PMO-1 (targets positions 11-23 & 91-103 and is 26 bases long), PMO-4 (targets positions 21-33 & 101-113 and is 26 bases long) and PMO-6 (targets positions 21-33 & 101-113 and is 26 bases long). Each one of these PMOs induced exon skipping:



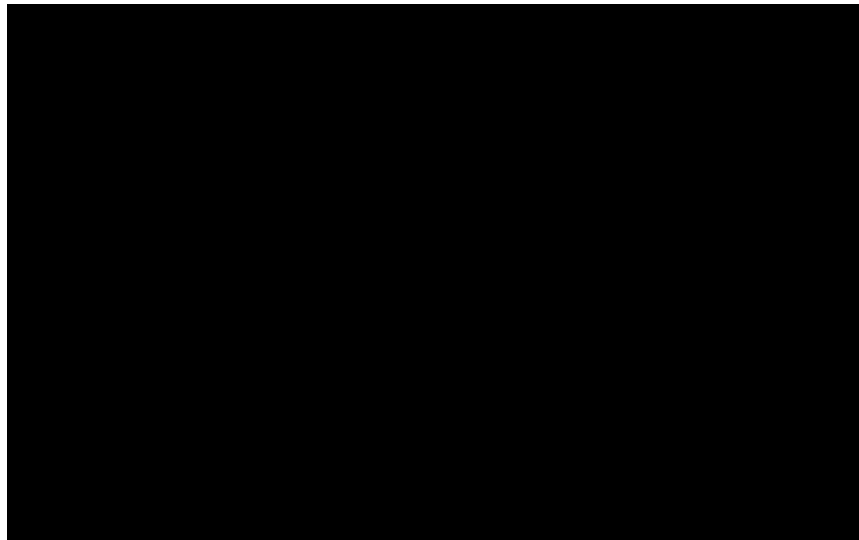
45. U.S. Patent Publication No. 2023/0097387 (to NS) discloses weasel PMOs targeting exon 51. As disclosed in Table 3, all of the weasels are 30 bases or less in length. Moreover, many weasels target regions that sit more than 100 bases apart. *See* Table 3 at *e.g.*, PMOs 65-89.

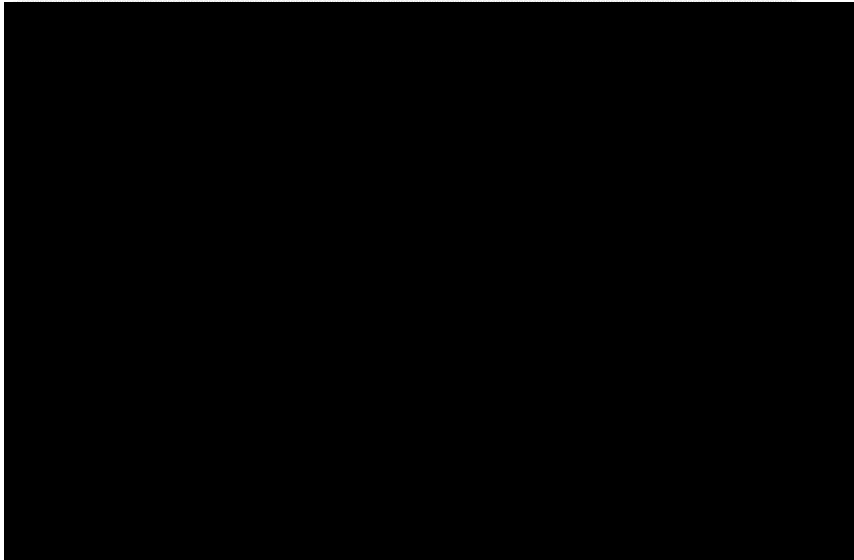
65	H51_8-20_128-141	27	CGGTAAGTTCTGTACAGTAACAGTCTGA	8987.11	8987.52	65
66	H51_4-16_128-141	27	CGGTAAGTTCTGTACAGTCTGAGTAG	9003.11	9003.22	66
67	H51_6-17_127-141	27	CGGTAAGTTCTGTCCACAGTCTGAGT	8963.09	8963.28	67
68	H51_5-15_128-142	26	TCGGTAAGTTCTGTCCAGTCTGAGTA	8638.98	8638.76	68
69	H51_5-16_129-142	26	TCGGTAAGTTCTGTACAGTCTGAGTA	8663.99	8633.39	69
70	H51_6-16_128-142	26	TCGGTAAGTTCTGTACAGTCTGAGT	8638.98	8638.34	70
71	H51_5-17_129-142	27	TCGGTAAGTTCTGTAAACAGTCTGAGTA	9002.11	9002.21	71
72	H51_6-17_128-142	27	TCGGTAAGTTCTGTCAACAGTCTGAGT	8978.10	8978.75	72
73	H51_6-18_129-142	27	TOGGTAAGTTCTGTAAACAGTCTGAGT	8993.10	8993.63	73
74	H51_2-12_128-142	26	TCGGTAAGTTCTGTCTGAGTAGGA	8678.99	8679.82	74
75	H51_2-11_128-142	25	TCGGTAAGTTCTGTCTGAGTAGGA	8348.74	8348.88	75
76	H51_-1-13_131-142	26	TCGGTAAGTTCTGTCTGAGTAGGAGC	8704.00	8703.95	76
77	H51_5-15_128-141	25	CGGTAAGTTCTGTCCAGTCTGAGTA	8308.87	8308.76	77
78	H51_4-13_131-142	22	TOGGTAAGTTCTGTCTGAGTAG	7339.53	7339.90	78
79	H51_-3-7_128-142	25	TCGGTAAGTTCTGTCTGAGTAGGAGCTA	8348.88	8348.60	79
80	H51_-2-9_129-142	25	TCGGTAAGTTCTGTGAGTAGGAGCT	8388.89	8388.22	80
81	H51_2-11_127-141	25	CGGTAAGTTCTGTCCCTGAGTAGGA	8333.88	8333.90	81
82	H51_-2-13_127-138	27	TAAGTTCTGTCCGTCTGAGTAGGAGCT	8994.10	8994.30	82
83	H51_-7-7_128-141	28	CGGTAAGTTCTGTCTGAGTAGGAGCTAAAAT	9366.24	9366.55	83
84	H51_-3-11_120-133	28	TCTGTCCAAGCCCGCTGAGTAGGAGCTA	9288.21	9288.36	84
85	H51_6-18_128-141	27	CGGTAAGTTCTGTCTAACAGTCTGAGT	8978.10	8978.58	85
86	H51_-1-15_125-136	28	AGTTCTGTCCAACAGTCTGAGTAGGAGC	9327.22	9326.72	86
87	H51_-1-12_125-138	27	TAAGTTCTGTCCAATCTGAGTAGGAGC	8987.11	8987.28	87
88	H51_6-17_128-141	26	CGGTAAGTTCTGTCAACAGTCTGAGT	8647.99	8648.53	88
89	H51_6-18_130-142	26	TCGGTAAGTTCTGTAAACAGTCTGAGT	8662.99	8663.19	89

46. Many of these PMOs induced exon skipping. A handful of those AOs are shown below:



47. U.S. Patent No. 10,144,931 (to NS) discloses weasel PMOs targeting exon 45. All of the weasels disclosed in Table 1-1 are 30 bases or less in length. *See* Sequence Listing. Moreover, many weasels target regions that sit more than 100 bases apart and were effective at exon skipping:





48. Finally, International Publication No. WO 2020/089325 (to Biomarin Technologies B.V.) discloses that “linking two AONs of the invention can lead to compounds that show improved characteristics for possible treatment of genetic disorders such as DMD,” *i.e.*, it discloses making weasels. WO 2020/089325 at 3:20-22. WO 2020/089325 states “[p]referably said first and second antisense oligonucleotides of the compound of the invention each have a length of 8 to 37 nucleotides, more preferably of 10 to 33 nucleotides, even more preferably of 16 to 22 nucleotides, most preferably of 18 to 22 nucleotides.” WO 2020/089325 at 9:17-21. Thus, according to Biomarin, the total length of the weasel AO can be as short as 16 bases in length.

### **3. Dr. Dowdy’s Other Issues Regarding the CERI Experiments**

49. CERI Report bearing study number 0643 tested four AOs labeled ASO-1, ASO-3, ASO-4, and ASO-5. ASO-2 was designed to target nucleotides +45+62 and was tested in a pilot experiment. I understand that the Final Reports for the pilot experiments have been produced as NS00153927 and NS00153963.<sup>23</sup> The pilot experiment tested ASO-2, in addition to ASO-1, as

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<sup>23</sup> The objective of the pilot experiments described in NS00153927 was “[t]o examine cell culture, differentiation, transfection, and gene expression analysis for assessment of gene expression level

[REDACTED]

potential positive controls. Obviously, only one positive control was necessary for the actual experiment, and a decision was made to use ASO-1 (+39+69), which was likewise identified in the UWA Patents as inducing skipping (SEQ ID NO: 193).

50. CERI Report bearing study number 0644 tested [REDACTED]  
[REDACTED] was tested in a pilot experiment. Again, the pilot experiment tested [REDACTED] as potential positive controls. As only one positive control was necessary for the actual experiment, a decision was made to use only [REDACTED] which was likewise identified in the UWA Patents as inducing skipping (SEQ ID NO: 192).

51. CERI Report bearing study number 0661 tested [REDACTED]  
[REDACTED]. I do not have an explanation why CERI labeled some PMOs with an “R,” although they do correspond to the “experimental” PMOs I selected. I also do not have an explanation why CERI’s labeling seemingly skipped the numbers [REDACTED]  
[REDACTED]

52. Finally, other than the pilot experiments and the experiments described in the three final CERI reports, I confirm that no additional test of exon 53 AOs were conducted by CERI under my direction. I further confirm that any interim test results provided by CERI were not inconsistent with or otherwise changed as compared to the results described in the three final CERI reports.

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in myotube exposed antisense oligomer.” NS00153929. The objective of the pilot experiments described in NS00153963 was “[t]o examine transfection conditions for assessment of gene expression level of in myotube exposed to morpholino antisense oligomers (PMOs).” NS00153965.

**IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE**

NIPPON SHINYAKU CO., LTD., Plaintiff,	)	
	)	
v.	)	C.A. No. 21-1015 (JLH)
	)	
SAREPTA THERAPEUTICS, INC.,	)	<b>DEMAND FOR JURY TRIAL</b>
Defendant.	)	
SAREPTA THERAPEUTICS, INC. and THE	)	<b>FILED UNDER SEAL</b>
UNIVERSITY OF WESTERN AUSTRALIA,	)	
Defendant/Counter-Plaintiffs,	)	
	)	
v.	)	
	)	
NIPPON SHINYAKU CO., LTD. and NS	)	
PHARMA, INC.,	)	
Plaintiff/Counter Defendants.	)	

**NIPPON SHINYAKU CO., LTD. AND NS PHARMA, INC.’S REPLY IN SUPPORT OF  
THEIR MOTION *IN LIMINE* NO. 1 TO PRECLUDE IMPROPER RELIANCE ON  
POST-PRIORITY DATE EVIDENCE TO SUPPORT THE UWA PATENTS’ VALIDITY**

Amanda S. Williamson (admitted *pro hac vice*)  
Christopher J. Betti (admitted *pro hac vice*)  
Krista V. Venegas (admitted *pro hac vice*)  
Wan-Shon Lo (admitted *pro hac vice*)  
Maria E. Doukas (admitted *pro hac vice*)  
Zachary Miller (admitted *pro hac vice*)  
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*Attorneys for Plaintiff/Counterclaim Defendant  
Nippon Shinyaku Co., Ltd. and Counterclaim  
Defendant NS Pharma, Inc.*

**Dated: April 29, 2024**

Sarepta misstates the law on post-priority date evidence. Such evidence is relevant only to show invalidity under 35 U.S.C. § 112. *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1374-75 (Fed. Cir. 2017). With respect to enablement, the proponent must show that the subsequent experimentation was an attempt to make additional species or followed the specification's teachings.<sup>1</sup> *Amgen Inc. v. Sanofi*, No. 14-1317-RGA, 2019 WL 4058927 at \*13-14 (D. Del. Aug. 28, 2019) ("*Amgen II*"). Otherwise, the evidence arises "in a subsequent state of the art" and is irrelevant to enablement. *Id.* at \*14; *see also Trs. of Boston Univ. v. Everlight Elecs. Co.*, 896 F.3d 1357, 1363 (Fed. Circ. 2018) (post priority date evidence "not probative" of enablement because no evidence that "results were accomplished by following the specification's teachings"); *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 126 F. Supp. 2d 69, 162 (D. Mass. 2001) (noting post-filing date publications showing enablement "us[ed] the techniques taught in the Amgen specification"). Sarepta cannot meet that burden here; its own expert testified that post-priority date efforts were independent and did **not** focus or rely on the "hot spot" purportedly identified by the UWA Patents. NS MIL #1, Ex. 1 at 192:18-193:18 (researchers' "independent analysis" screening the entire exon "confirmed" or "independently identified" the hot spot). Evidence that other researchers' independent work identified functional species not disclosed in the specification is irrelevant to establishing enablement and written description. Sarepta should not be allowed to imply or argue otherwise in any context. The risk of confusing and misleading the jury is too great.

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<sup>1</sup> Sarepta mischaracterizes the court's February 14, 2019 decision in *Amgen II*, which in fact supports NS's arguments. There, the court **granted**-in part *Amgen's* motion to **exclude** post-priority date evidence as irrelevant to lack of enablement, but **denied** the motion, *i.e.*, allowed defendants to rely on that evidence, as to lack of representative species. *Amgen II*, 2019 WL11071409 at \*1-2 (discussing Amgen's MIL # 3). The court also **granted** Defendants' motion to **preclude Amgen** from testifying that post-priority date antibodies and data were explicitly disclosed in a priority application. *Id.* at \*3 (discussing Defendants' MIL #1). After trial, the court reaffirmed its decision as to Amgen's MIL #3 because Sanofi failed to establish that evidence was relevant. *Amgen II*, 2019 WL 4058927 at \*13-14.



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Respectfully submitted,

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